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COLLECTED STUDIES

FROM THE

BUREAU *of* LABORATORIES

(Foot of East 16th Street)

CITY OF NEW YORK

Dr. WILLIAM H. PARK, Director

VOLUME VIII

1914-1915

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*Died Jan. 1st, 1916

PREFACE TO VOLUME VIII

The reports appearing in the present volume include studies carried on during the years 1914 and 1915, in the Divisions (Bacteriological, Preventive Medicine, Chemical) of the Bureau of Laboratories.

Many of the reports in this volume have already appeared in print, a few are in press, and the remaining ones are published only in this collection.

The statistical matter of the Bureau of Laboratories will be found, as in previous years, in the Annual Reports of the Department of Health.

The recipients of this volume will confer a favor upon their colleagues of the Bureau of Laboratories by acknowledging receipt, and by sending their publications in exchange. These should be addressed to the Librarian, Bureau of Laboratories, foot of East Sixteenth street, New York City.

W. H. P.

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SUMMARY OF FOUR YEARS OF CLINICAL AND BACTERIOLOGIC EXPERIENCE WITH MENINGITIS IN NEW YORK CITY

PHEBE L. DUBOIS AND JOSEPHINE B. NEAL

(*Reprinted from the American Journal of Diseases of Children, January,* 1915. *Vol. IX, pp.* 1-16)

July 1, 1910, the preventive medicine annex of the Research Laboratory of the New York Board of Health took over the preparation, distribution and administration of antimeningitis serum from Rockefeller Institute. Jochmann, Kolle and Wassermann, the New York Board of Health and others had prepared the serum some time before it was taken up by Rockefeller Institute, so the serum itself was not new, but the credit for introducing its intraspinal use in this country is Dr. Flexner's. Under his direction, the Rockefeller Institute prepared it and distributed it to all who would supply careful records. This was of the greatest advantage in procuring a quick decision as to its great value. During part of the last big epidemic here (1904-05) the Health Department tried using it subcutaneously and found it to be of such doubtful value that its preparation was discontinued.

Meningitis is a reportable disease in New York City. The plan of the department from the first has been to have the physician on each case, so soon as he is known by the report of the case, offered the services of a department consultant.

We make our appointments with the attending physicians for the earliest moment possible and see cases nights and Sundays with them, because occasionally twelve hours' delay will seem to be a possible cause of a fatal outcome. We go over the history, examine the case, and if it seems to us to warrant it, even though we may not consider it to be one of meningitis, we do a lumbar puncture. It takes tact and infinite patience to persuade some of the poor, ignorant, foreign parents to allow us to do it, but usually if properly approached, they finally give in.

The conditions we have to consider mainly are epidemic or meningococcic meningitis, other purulent meningitides due most commonly in our experience to the streptococcus and pneumococcus and less commonly to the *Streptococcus mucosus capsulatus* and influenza, tuberculous meningitis, poliomyelitis and meningism, particularly in pneumonia. We see many other conditions, as our yearly tabulations will show—sufficient, indeed, to make our work pleasantly exciting, for when a case is reported the probabilities are that it is not going to be epidemic cerebrospinal meningitis.

As a general thing, we do not go to see patients in hospitals. At first we thought such cases received proper treatment and did not need us, but as our knowledge of meningitis has increased and we have from time to time had occasion to observe such treatment and the results, we think hospitals would be wise to call us in once in a while to instruct the house staffs, for they are often left pretty much to their own devices, and though the serum treatment of meningitis is beginning to find its way into text-books the busy attending physicians have not had much occasion to learn about it and have little advice to offer.

The points of differentiation in their order of value among the above named conditions are in general, lumbar puncture with the examination of the fluid, the history and the physical signs.

A clear fluid increased in amount indicates usually one of the following conditions: tuberculous meningitis, poliomyelitis, syphilitic involvement of the central nervous system, brain tumor or meningismus. A cloudy fluid is the result of a meningitis due to the meningococcus or some of the other pyogenic organisms.

In examining fluids, we make both bacteriologic and chemical studies and when these are not conclusive inoculate guinea-pigs. We make smears and cultures from the sediment if fluids contain any. Other fluids are centrifuged for one hour at high speed and the smear made by putting the last few drops from the tube onto a slide and evaporating to dryness in the incubator. Smears from cloudy fluids are stained by Gram's method, those from clear fluids with Ziehl's tubercle bacillus stain. Occasionally it is necessary to make a capsule stain. In examining the smears we note the presence or absence of bacteria and the number and kinds of cells.

We have never counted the cells for two reasons: first, it is some time usually between the time fluids are withdrawn and the time that they are examined, for most cases are at some distance from the laboratory; secondly, we see no great advantage in doing it. We can tell from the stained sediment whether the cells are increased much, little, or not at all, and there is nothing particularly diagnostic in the exact number of cells per cubic millimeter. Moreover, since in most cases the cells are very few in number and it is impossible to use a large amount of fluid, no method can be very accurate. In poliomyelitis at the stage at which we usually see it, and in tuberculous and syphilitic meningitis, the cells are considerably increased, there being from 20 to 30 to a field. Mononuclears predominate up to 95 per cent. often. In epidemic cerebrospinal meningitis and the meningitides due to other pyogenic organisms the cells are enormously increased, so much so that it is not necessary to centrifuge. The polymorphonuclear leukocytes predominate, often up to 98 per cent. at first. Later, if the condition improves, the cells decrease and gradually the percentage of mononuclears increases.

The cultures are made after the smears are examined. Those showing *Streptococcus pyogenes, Streptococcus mucosus capsulatus* or meningococcus, or those in which no organism is found, but one of these is suspected of being the cause, are planted on 2 per cent. glucose ascitic agar. Those showing influenza or pneumococcus are planted on blood agar. Clear fluids are always injected subcutaneously in from 5 to 7 c.c. amounts into the groins of a guinea-pig. The pig is kept for a month and then injected in the axilla with 1 c.c. of crude tuberculin diluted to 3 c.c. with normal saline. Usually if it is tuberculous it is dead next morning. For proof of tuberculosis we depend on caseous inguinal glands or tubercles in the spleen or both at necropsy. Clear fluids that have become contaminated will sometimes produce enlarged inguinal glands that may even go on to caseation, but 1 c.c. of tuberculin will not kill in those cases.

We make chemical tests for albumin, globulin and reduction with Fehling's solution. For albumin, we use the nitric acid ring test and judge of the amount of albumin by the depth of the ring when it is first formed. The globulin test is that of Noguchi and is made by adding to one part of the fluid four or five parts of 10 per cent. butyric acid made up in normal saline, boiling, adding about as much normal sodium hydroxid as there was fluid to begin with and boiling again. Too much sodium hydroxid spoils the test by redissolving the globulin. Normal fluids will give a very faint opalescence; pathologic fluids a flocculent precipitate varying in amount with the severity of the inflammation present. Fluids showing globulin are said to be the result of inflammation of the meninges and we have never had reason to doubt it. Indeed, to us it is the most important of the chemical tests, because it separates fluids due to meningism from those due to a true meningeal infection. Both the albumin test and the globulin test are of course worthless, if there is blood present. The majority of clear fluids reduce Fehling's solution. The statement is now and then made that a differential point in diagnosis between tuberculous meningitis and poliomyelitis is the fact that poliomyelitis fluids will reduce Fehling's solution, but tuberculous fluids will not. We have tried Fehling's reaction with eighty-eight known tuberculous fluids and sixty-five, or 73 per cent., gave good reduction. So, while the absence of reduction is of marked significance, the presence of reduction means absolutely nothing. The fluid from a case of heat prostration also failed to reduce Fehling's. In cloudy fluids it is not uncommon to find the reduction present if the case is seen early and is mild, then to go through a period where there is no reduction, and as the patient improves and the fluid clears, to have it return. In making the test we use equal quantities of Fehling's solution and spinal fluid.

We have examined two fluids and have seen a third that illustrated the syndrome of Froin. This is characterized by spontaneous coagulation and a yellow color. The pathogenesis of this condition is not known except, of course, that some hemorrhage has taken place. The two fluids we examined were from poliomyelitis patients, one of whom recovered and one died. The third fluid was from a patient with epidemic cerebrospinal meningitis supposed to have had a fracture of the skull. This patient recovered.

Table 1 is a concise statement of the various characteristics of the different fluids.

From July 1, 1910, to July 1, 1914, we examined 992 cerebrospinal fluids. Included with those we withdrew are some sent in by physicians who made their own punctures. Our method of examination and the differential points have already been discussed. We have found that examinations of fluids from epidemic cerebrospinal meningitis are more apt to be successful if done promptly, as the meningococcus sometimes autolyzes in twelve hours so that they will not grow and are very difficult to distinguish by smear. As cases recover the organisms are found more and more within the cells, and they usually show in spreads after the cultures become negative.

In examining for tubercle bacilli, we find the sediment after prolonged centrifuging at high speed better adapted to our purposes than the examination of the fibrin web. As our fluids are brought to the laboratory from varying distances, the formation of the web is disturbed; frequently, too, we wish to examine a fluid without allowing time for the web to form. By this method we get positive results in about 65 per cent. of cases. This is not nearly so high a percentage as that obtained by Dr. Hemingway at the Babies' Hospital using the web, but she was able to make several punctures, if necessary, while we rarely see a case of tuberculous meningitis more than once.

The history is of importance in the purulent meningitides, for, though they are differentiated from each other only by microscopical and cultural examinations, a history of middle-ear disease or fracture of the skull makes us suspect that some other than the meningococcus is the infecting organism. In differentiating between tuberculous meningitis and poliomyelitis the history is of more importance than the examination of the spinal fluid if tubercle bacilli are not found, because the chemical and microscopic pictures are so frequently identical in the two conditions. In poliomyelitis there is usually the history of a sudden onset with a very high temperature dropping quickly. There may have been prodromata; gastro-intestinal disturbance is common, and many cases show redness of the throat or some involvement of the upper respiratory tract, but they are so slight that it takes careful questioning to elicit them. The history of a tuberculous case, on the other hand, is usually that of an insidious onset preceded by unusual irritability, though occasionally we see children in families in which they have been carefully observed that give a history of a sudden onset, frequently with convulsions. Usually the temperature has been between 99° and 101° F.; occasionally it will have been normal or subnormal. Transient paralyses, projectile vomiting and crying out in the night are frequently mentioned. Given a patient in apparent coma, usually a determined effort will rouse the child with poliomyelitis and he will answer intelligently, though afterward he sinks back immediately into stupor. It is impossible to rouse the tuberculous child in the stuporous stage.

The differentiation between epidemic cerebrospinal meningitis and tuberculous meningitis is usually comparatively easily made. Cases of epidemic cerebrospinal meningitis usually give a history of a sudden onset as against a slower onset in tuberculous meningitis. The patient's mental condition usually remains good in meningococcic meningitis unless it is a fulminating case. In tuberculous meningitis the history is commonly that of irritability followed by increasing stupor from which it is impossible to arouse the child. Patients with epidemic cerebrospinal meningitis show marked rigidity of the neck; tuberculous patients are apt to have slight or moderate anterior-posterior rigidity, but none laterally. In infants the rigidity of epidemic cerebrospinal meningitis is so easily overcome as to make one often doubt its existence, but when the infant is turned on the side the head is markedly retracted. The fever is higher in epidemic cerebrospinal meningitis, and runs an irregular course. Projectile vomiting is common in tuberculous meningitis and rare in epidemic meningitis.

Very few luetic or other chronic conditions of the central nervous system or meninges come to our attention, but we do see a good many cases of meningism. By meningism we mean that condition in which meningeal symptoms arise in the course of some disease, the cerebrospinal fluid being increased in amount but normal in character. Some difference of opinion exists in regard to the use of the term. It was introduced by Dupré, who considered it a functional disturbance. Other writers have used the term serous meningitis, pseudomeningitis, meningitis sine meningitide and meningitis infectiosa circumscripta or circumscribed meningitis. This latter term is considered preferable by Plaut, Rehm and Schottmüller. Holt describes a case which showed at necropsy a circumscribed meningitis. Huber in an article on "Pneumococcus Meningitis and Meningism," inclines to the opinion that the pathologic basis is a localized inflammation, usually of infectious origin.

In the face of this evidence we cannot deny that cases exist in which the clinical picture and the increased fluid is due to a circumscribed infection, but from our experience it seems to us highly improbable that this is always or even often the case.

We have seen 80 cases of which the outcome in 53 was recovery and in 22 death, while with 5 the outcome is unknown. The diseases which the meningism accompanied are as follows:

Pneumonia—uncomplicated	39
Gastro-intestinal disease	8
Whooping-cough (two patients had pneumonia also)	6
Scarlet fever (one patient had pneumonia also)	5
Typhus	3
Typhoid complicated by pneumonia	1
Measles	1

Middle-ear disease	1
Retropharyngeal abscess	1
Orbital abscess	1
Pott's disease	1
Epilepsy	1
Rachitis	1
Streptococcus osteomyelitis	1
Staphylococcus septicemia	1
Influenza	1
Nephritis	1
Heat prostration	1

Of the 22 patients who died, 8 died within twenty-four hours, and were desperately ill when seen. Of the 14 living longer than twenty-four hours, 4 died of pneumonia (in 1 two punctures were made with normal fluid in each instance), 1 of gastro-intestinal disease, 1 of whooping-cough complicated with bronchopneumonia, 3 of scarlet fever (1 complicated by bronchopneumonia and diphtheria, and 1 very septic), 1 of typhoid complicated by pneumonia, 1 of staphylococcus septicemia (two punctures done with normal fluid), one of streptococcus osteomyelitis (necropsy showed normal brain and meninges), 2, cause unknown.

In most of these cases the meningeal symptoms did not progress. Had they done so, a second puncture would have been made. In two cases, as noted, second punctures were done revealing normal fluids. In all cases the severity of the accompanying disease or diseases was quite sufficient to account for death. Of course, this by no means proves the absence of a localized meningeal infection. We have never seen a case of generalized meningitis following a case of meningism, however, which we should expect to see occasionally considering the number under observation, were there a localized infection of the meninges, and in view of the relatively large number of patients that recover promptly, we believe that in most cases the condition is a functional one, probably of toxic origin.

In sixty-nine of these cases lumbar puncture was done. In the other cases we did not do a puncture, either because the symptoms had cleared up considerably when the case was seen or because with the accompanying disease we felt sure the case was one of meningism and advised waiting. Latterly, we have done a lumbar puncture even though we have felt sure it was meningism, as the withdrawal of the fluid seems to hasten recovery.

Of course, the amount of fluid depends on the stage of the condition at which the puncture is done. We have withdrawn very large amounts—from 80 to 100 c.c. In other cases the amount has been little increased above normal. It is commonly accepted that in a true meningitis the fluid is inflammatory in character—of the nature of an exudate. Such a fluid shows an increase in albumin and globulin and in the number of cells. In a meningism, as we use the term on the other hand, the fluid is of the nature of a transudate. Except in the cases noted below, the cells have not been increased, neither has the albumin and globulin content, and Fehling's has been readily reduced. In all cases they have been negative bacteriologically, by smear culture and animal inoculation.

In two cases of pertussis, the patients dying the same day the puncture was done, there was some increase in albumin and globulin and number of cells, in one case about equally divided between endothelial cells and polynuclears, in the other 95 per cent. or more polynuclears. These changes were presumably ante mortem. In one case of typhus the cells were increased—95 per cent. mononuclears. This patient recovered. The case of heat prostration showed a faint increase in albumin and a slight increase in cells, 70 per cent. being polynuclears. This fluid, as has been stated before, failed to reduce Fehling's, though repeated tests were made, and was the only fluid that did not do so readily. The patient recovered.

Plaut, Rehm and Schottmüller include under the term "meningism" cases with spinal fluid in which there is an increase in albumin and globulin and in number of cells usually mononuclear, sometimes polynuclear. We feel that such instances may be mild and recovering cases of epidemic cerebrospinal meningitis in which the organism has cleared up before the puncture was made. We have seen several cases ourselves in which from the clinical history, the progress of the case and the character of the fluid we were convinced of the diagnosis, while we were unable to prove it bacteriologically.

In examining patients, we particularly note the following points: the presence or absence of Macewen's sign or in infants a bulging fontanelle; the eyes (whether or not the pupils are dilated, contracted or unequal, presence of conjunctivitis, strabismus, nystagmus, etc.); the mental condition (whether there is delirium, irritability or stupor); the presence or absence of Kernig's and Brudzinski's signs; regularity in rate and depth of respiration and rate and volume of pulse; the reflexes (whether they are increased, diminished or absent); the temperature and presence or absence of an eruption. Of these signs we consider the stiffness of the neck, the variation of regularity in rate and depth of respiration and Macewen's and Brudzinski's signs the most important. Macewen's sign consists of a change from the normal in the percussion note over the lateral ventricles due to increased intraventricular pressure. It takes some practice to appreciate it. Brudzinski's sign consists in the flexion and eversion of the legs and arms when an attempt is made to flex the head on the chest. Wherever we have found a Macewen we have always obtained increased fluid, except in cases of basic meningitis, in which case the connection between the brain and cord is partially or totally cut off.

The mental condition is frequently good in epidemic cerebrospinal meningitis unless the case is of the fulminating type. Early in tuberculous meningitis there is irritability, later stupor. In poliomyelitis irritability not followed by stupor is the usual thing.

Conjunctivitis is fairly common in epidemic cerebrospinal meningitis and rare in other meningeal conditions. Ptosis and strabismus are more common in tuberculous meningitis, but may occur in other meningeal conditions.

We do not attach much significance to Kernig's sign, because it is difficult to make sure of it in young children. Brudzinski's sign is of much greater value to our minds.

In true meningitis the depth and time between respirations is markedly irregular, the so-called Biot's breathing. Late, particularly in tuberculous meningitis, the respiration frequently becomes Cheyne-Stokes. The pulse is more apt to be irregular in rate and volume in tuberculous meningitis than in other meningeal conditions.

Palsies are uncommon in epidemic cerebrospinal meningitis; in 112 cases we have seen palsy twice only. It is common, but usually transitory in tuberculous meningitis, and always present in frank cases of poliomyelitis.

As has already been stated, the temperature in general is low and long continued in tuberculous meningitis, is high, rising rapidly and dropping quickly in poliomyelitis and runs a very irregular course in epidemic cerebrospinal meningitis.

Eruptions aside from herpes are not very common in our experience, though in times past epidemic cerebrospinal meningitis has been known as spotted fever. In 112 cases we have seen an eruption sixteen times. In

TABLE 1

Characteristics of the Various Fluids

Meningeal Condition	Pressure	Amount c. c.	Appearance	Cytology	Bacteriology	Albumin	Globulin	Fehling's Solution	Animal Inoculation
Normal.........	Normal	5-10	Clear	Very few cells	Sterile	±	±	+	Negative
Meningismus.....	Increased	10-100	Clear	Very few cells	Sterile	±	±	+	Negative
Poliomyelitis.....	Increased	20-100	Clear; sometimes slight fibrin web	Early polynucleosis; later lymphocytosis up to 95 per cent.;—endothelial cells	Sterile	+−++	+−++	+	Negative
Tuberculous meningitis	Increased	30-120	Clear fibrin web	Lymphocytosis up to 95 per cent.	Tubercle bacilli	++−+++	++−+++	− in 25 per cent.	Tuberculosis in four weeks
Epidemic cerebrospinal meningitis	Increased	5-120	Cloudy	Polynucleosis up to 98 per cent.	Meningococcus	++−++++	++−++++	+ or − according to severity and stage	
Meningitis due to other organisms	Increased	20-100	Cloudy	Polynucleosis up to 98 per cent.	Infecting organism	++−++++	++−++++	− may be + early	

cases in which it did occur there was nothing characteristic about the eruption itself, its location or the time of its appearance or disappearance.

Table 2 shows the number of cases from July 1 of one year to July 1 of the next year for the last four years.

The increase in numbers from year to year has been partly due to the gradual spread of the knowledge that such work is being done. We regret that even after four years there are many physicians in New York who are entirely unaware of the existence of our department. Aside from that, we feel that there has been a real increase in the number of cases of epidemic cerebrospinal meningitis. It is a disease for which apparently the majority of individuals have a natural immunity. Many healthy people carry meningococci in their noses and throats for long periods of time, acquiring them either from cases or from carriers. After a certain length of time, when there has been little meningitis about, a new generation of susceptible individuals comes on the scene. These, with possibly certain meteorologic factors which we do not understand, probably determine epidemics to a large extent. It has been about ten years since the beginning of the last epidemic. With the increasing number of cases another epidemic may be approaching. Fortunately, this last year it has been of a mild type.

TABLE 2.

NUMBER OF CASES BY YEARS, JULY 1, 1910, TO JULY 1, 1914.*

	1910-1911	1911-1912	1912-1913	1913-1914	Total
Epidemic cerebrospinal meningitis	17	25	29	41	112
Tuberculous meningitis	22	44	37	51	154
Other meningitides	11	7	11	18	47
Anterior poliomyelitis	1	25	16	5	47
Pneumonia	6	8	16	22	52
Other diseases	14	30	20	53	117
	71	139	129	190	529

* Some few of the cases during the first two years were seen only by Dr. Sophian, who had charge of the work during that time.

TABLE 3.

NUMBER OF CASES DURING THE FOUR QUARTERS OF THE YEAR.

	July-October	October-January	January-April	April-June
1910-1911	..	5	3	9
1911-1912	6	4	10	5
1912-1913	4	6	9	10
1913-1914	2	4	16	19
	12	19	38	43

Table 3 gives the number of cases during the four quarters of each year. The greatest number of cases occurs between March 1 and June 1 each year.

Table 4 shows the number of physicians with whom we have consulted and the number of cases we have seen with each.

There are 531 instead of 529 cases, because in two instances there were two physicians in charge of cases, and in order to make the number of physicians come out right it was necessary to count each of these cases as two.

TABLE 4.

Physicians Consulted and Cases Seen.

Physicians	No. of Cases Seen with Each	No. of Cases Seen
254	1	254
60	2	120
10	3	30
8	4	32
8	5	40
4	6	24
1	7	7
3	8	24
348		531

The treatment of epidemic cerebrospinal meningitis resolves itself into prophylaxis and specific and general treatment. The prophylaxsi consists in quarantining patients ill with the disease and those in contact with them who show meningococci in cultures from the nose and throat. Under ordinary circumstances epidemic meningitis is not very highly contagious, but it sometimes becomes so. We have seen more than one case in a family in only four instances. The first time a child of 11 had it and three months later her mother had it. Both recovered. The second time two brothers, adults, were sick in the same room. Most insanitary conditions prevailed. The first brother had been ill two weeks and had had no doctor. The second was a fulminating case and the patient died in three days. The first one was sent to a hospital and finally died after five weeks' illness. In the third instance, a brother and sister, adults, and the 8-year-old daughter of the sister were all ill in the same apartment. The brother and sister were taken ill within two hours of each other and both died. The little girl was taken five days later and recovered. The day before the little girl was taken sick a neighbor, a man living in the same house, came down with it and eventually recovered. The fourth instance was that of a sister of 14 and a brother of 3½. The sister had been taken sick two days before the boy. The attacks were mild and both recovered.

The question of carriers is rather a difficult one. In the first place, it requires careful bacteriologic technic to isolate meningococci from nose and throat cultures when they are few in numbers, and the work cannot be left to laboratory assistants unless they have exceptional training. When there are many cases to be diagnosed and treated is the time when nose and throat cultures from carriers come in thick and fast, and until quite recently we have not had a sufficiently large force to manage the cultures satisfactorily. From some work done here two years ago, we think the most satisfactory method of dealing with carriers is to swab out the nose and throat two or three times daily with 20 per cent. argyrol. We hope to do more work along this line this winter. Hatchel and Hayward report success with a spray of antimeningococcic serum and with subcutaneous injections of meningococcus vaccine.

The specific treatment consists in the intraspinal administration of antimeningitis serum. This is a specific immune serum of therapeutic value only in meningococcic meningitis and then only when administered subdurally. In making a lumbar puncture we do not use either general or local anesthesia. We think that general anesthesia is dangerous, and local anesthesia takes so much preparation and time that the overcoming of the pain does not compensate for the increased nervous tension on the part of the patient. Adults not acutely ill who submit to lumbar puncture for diagnostic purposes do not seem to mind much. The back is not very sensitive, and if the patient is held properly—lying on the side with the knees drawn up against the abdomen, the neck bent and the back well arched so that the intervertebral spaces will be as great as possible—and the operator is skillful there is very little pain. Never under any circumstances do we do it with the patient sitting up. Iodin is used over about 4 square inches immediately around the point of election for puncture and a sterile or bichlorid towel is laid over the hips through which to find the landmarks. We have never to our knowledge had a secondary infection of the meninges of the cord. Sometimes the skin has become infected in small children from soiled napkins. We use a Quincke needle size 18 or 19, and go in the mid-line through the notch most nearly coinciding with a line drawn from crest to crest of the ilium. A piece of tubing about 15 inches long is attached to the metal connection that fits in the end of the needle when the stylet is withdrawn. To the other end of the rubber tubing is attached the barrel of a syringe. We usually cut the rubber and insert a short piece of glass tubing near the metal connection so that we can see the fluid flowing out or the serum flowing in. It is well to attach the tubing in removing the fluid because by raising and lowering the glass container the rapidity of outflow can be regulated. A too sudden decrease in intracerebral pressure is undesirable. When the fluid withdrawn is cloudy we always inject antimeningitis serum at once even though we suspect that some other organism may be the cause. The serum does no harm no matter what the organism may be, and if it is meningococcic meningitis the earlier serum is administered the better. Later treatment depends on the examination of the cerebrospinal fluid. We have used streptococcus and pneumococcus serums in appropriate cases. No patient with pneumococcus meningitis directly under our supervision has recovered, but we know of two patients who did. One patient with streptococcus meningitis out of fifteen recovered, but no influenzal patient. At present we have a good supply of anti-influenzal serum furnished us by Dr. Flexner, and as cases come along we shall use it.

As stated above, if the first fluid is cloudy we inject antimeningitis serum. It is warmed to body temperature and injected very slowly by gravity under the least possible pressure. This method was introduced by Koplik. A syringe is dangerous, and is probably responsible for many deaths following the administration of serum. In general, the dose for an adult is from 20 to 40 c.c., and for infants and children from 3 to 20 c.c. The amount depends as much on the quantity of cerebrospinal fluid withdrawn as on the age. An infant will frequently stand 20 c.c. without difficulty. The dose should usually be at least 5 or 10 c.c. less than the amount of cerebrospinal fluid withdrawn.

We have seen a number of cases of undoubted dry taps during the course of cases of meningococci meningitis. The serum ran in freely and showed the usual variation in movements depending on respiration. In such cases it is advisable to proceed very slowly and to watch the patient carefully for the slightest change in pulse and respiration. We think that possibly in some cases the exudative period is followed for a short time by one of decreased secretion. At any rate, a dry tap is frequently followed by one in which fluid is obtained. In cases with thick exudate that will not flow through the needle, gentle suction with a syringe may be tried. If that fails a little serum injected will sometimes start the flow. In very severe cases we inject the serum every twelve hours until there is improvement. In moderate and mild cases we usually repeat it each day for the first four days. Further administration depends on the patient's general condition and the bacteriologic examination of the fluid. Usually from 4 to 6 injections are necessary, but we have employed as many as sixteen or more. We consider that we get better results if the patient is turned from side to side so that no two successive punctures are done with the patient lying on the same side. That insures the emptying of the lateral ventricles in rotation.

A number of times during or immediately after the injection of serum the patient has gone into shock. Respiration has become slow and shallow or ceased, the facies pale and pinched and the pulse rapid and thready. At first this was very alarming, but we have never had a patient die from it. If the needle is still in place we withdraw some of the serum. Artificial respiration is resorted to if breathing has ceased and hypodermic stimulation is given for the heart. We have seen it happen much less frequently since we have been using smaller doses.

The serum we have been using lately contains 0.2 per cent. trikresol; earlier it contained 0.3 per cent. As is well known, the trikresol has been blamed by several physicians, especially Dr. Kramer of Cincinnati, for the fatal results that have been reported in a few instances following the injection of antimeningitis serum, usually in young children. Hale, of the Hygienic Laboratory at Washington, and Auer, of Rockefeller Institute, report experiments with dogs showing that serum containing trikresol is somewhat more toxic than unpreserved serum or that containing chloroform or ether. Auer carried on experiments with monkeys also, and showed that they were far less sensitive to trikresol serum than the dogs. Furthermore, in France, where serum without preservative is used, cases of shock and occasional fatal results are reported. As stated above, we have never seen such a case, although we have administered the serum considerably over five hundred times and to children of all ages, fourteen of our patients being under a year old. In view of our experience with serum that has always contained trikresol, we cannot believe that it is the cause of the fatalities. We think rather, that they may be due to injudicious administration of the serum—too large doses, or too rapid increase of pressure, which is likely to happen unless the gravity method is used—or to an unusual susceptibility on the part of the patient. On account of the fear of trikresol—unfounded, we feel—that has been produced by these reports, it seems advisable to try for a while the use of chloroform or a preservative. A comparison of the results with the serum prepared with the two different preservatives will enable us to decide which method is preferable.

We have not given the method of administering serum with the use of the blood-pressure apparatus a fair trial because we have so seldom had sufficient assistance to manage it. We think it is probably a help to those who have had little experience.

If a case shows a tendency to become chronic we make an autogenous vaccine and give it every four or five days in doses of from 250 to 1,000 million. Sometimes it has seemed to be very effective, but we have not had enough cases to be able to draw definite conclusions. We can say that we have never seen it do any harm. In treating cases it must be remembered that frequently the stiff neck is the last symptom to disappear and if the fluid has cleared up and the temperature stays down it may be entirely disregarded.

The use of hexamethylenamin (urotropin) comes between general and specific treatment. We recommend its administration in all acute meningeal infections. It is said that twenty minutes after its administration formaldehyd can be detected in the spinal fluid. It may not be there in sufficient amounts to do much good, but the procedure seems rational. The fact that meningococci may be isolated from the urine is another argument for its use. In one case of *Streptococcus mucosus capsulatus* meningitis we gave hexamethylenamin dissolved in normal saline intraspinally. The temperature dropped from 106 F. to 102 F., and the patient became rational, but the case terminated fatally.

In planning the general treatment we find it necessary to remember that epidemic cerebrospinal meningitis may be a greatly prolonged febrile disease. The patient is best kept in a quiet darkened room. Sedatives are needed if the patient is very restless. We have had one fatal prolonged case in which we thought the patient might have been saved had the doctor given her an opiate so that she might occasionally have had a few hours of rest. The bowels and bladder should receive careful attention, particularly the bladder. Retention and cystitis are not uncommon. The patient should be examined for a distended bladder daily and the family warned to report infrequency of micturition. Patients should not lie in a draught and should be carefully covered up, especially during and after puncture. We have learned by bitter experience how easily they fall victims to pneumonia. In ordering the hygiene of the sick-room it must be remembered that the meningococci are found in the secretions of the nose and throat and in the urine. The diet should be such that it may be easily digested but generous in amount. The high caloric diet of typhoid is indicated for the reason that meningitis, like typhoid, may be prolonged. The ice-bag gives a measure of relief for the headache.

Among the complications we have had:

Pneumonia in	7 cases
Basic meningitis in	6 cases
Paralysis in	3 cases
Hydrocephalus in	3 cases
Deafness in	3 cases
Cystitis in	2 cases
Serum rash in	2 cases
Iritis in	2 cases
Typhoid in	1 case
Scarlet fever in	1 case
Measles in	1 case
Purulent otitis media in	1 case
Blindness in	1 case
Arthritis in	1 case

In no case of basic meningitis under our care has recovery ever ensued. At first we used to try very hard to get the patients into hospitals to have brain puncture done. Now we explain to the family that it may do some good, but do not urge it strongly because we do not feel that it is of much avail. Of the cases with paralysis, two cleared up. One case of deafness cleared up entirely after the lapse of several months. The patients with typhoid and measles both recovered. We are much interested in the question of mental deterioration following meningitis and are following up our cured patients. In the course of time we shall have sufficient data from which to draw conclusions.

Table 5 shows our mortality statistics for the four years from July 1, 1910, to July 1, 1914.

TABLE 5.

MORTALITY, JULY 1, 1910, TO JULY 1, 1914.

Year	Total No. Cases	Patients Recovered	Patients Died	Result Unknown	Mortality Per Cent.
1910–1911	17	10	7	0	41
1911–1912	25	7	15	3	60
1912–1913	29	17	12	0	41
1913–1914	41	30	11	0	26

The 1911-1912 statistics need some explanation: four cases developed into basic meningitis and in five other fatal cases the patients were either in hospitals already or were sent there and we had slight oversight of the treatment.

We feel that this particular department—meningitis—is a worthwhile venture in treating cases of meningitis, in teaching physicians whom we see in consultation to administer serum and in training a group of experts who will be able to handle efficiently the outbreaks of meningitis that occur about every ten years in New York City.

STREPTOCOCCUS MENINGITIS WITH REPORT OF A CURED CASE

Phebe L. Du Bois and Josephine B. Neal

(*Reprinted from Archives of Pediatrics, January,* 1915)

On September 4, 1913, we were called to St. Vincent's Hospital to see a case of meningitis presenting the following history:

T. F., age twenty-six years, admitted to the hospital August 30th, complaining of very intense headache. Family and past history negative. On August 20th earache had begun following bathing in surf. August 23d went to New York Eye and Ear Infirmary, was told he had abscess of left ear and paracentesis was performed. He went to his home feeling pretty well, but the next day, August 24th, began to have headache. He went back two or three days later and was told he was all right. He came to St. Vincent's August 29th complaining of headache. Was given calomel and sent home. Was admitted August 30th.

Physical Examination.—Rigidity of neck, later opisthotonos; positive Kernig; knee-jerks present; pupils large, equal, react to light and accommodation; slight internal strabismus of both eyes, some nystagmus in left; pulse and respiration rapid; abdomen rigid; extremely hypersensitive; tremulous all over.

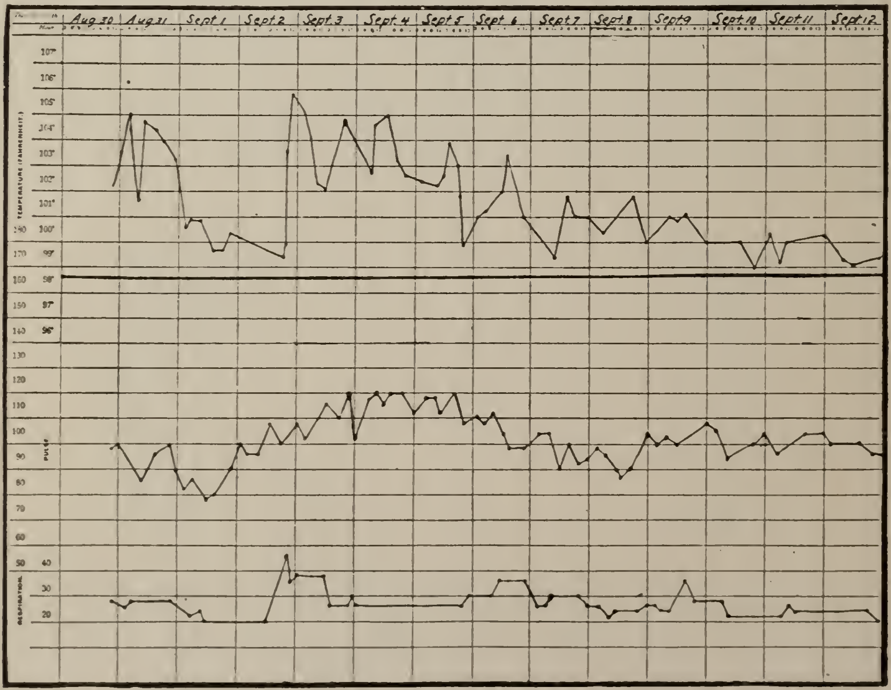

Fig. 1 Temperature, Pulse and Respiration Curves of T. T.

August 31st, lumbar puncture, 20 c.c. cloudy fluid under pressure; heavy sediment, globulin positive; cells increased, practically all polynuclears; no bacteria in smear or culture. Urine, trace of albumin, few hyalin casts.

September 1st, blood count, 14,500 white blood cells, 84 per cent. polynuclears.

September, 2d, blood count, 15,000 white blood cells, 86 per cent. polynuclears. Second lumbar puncture, 30 c.c. fluid.

September 4th, seen by meningitis department of Board of Health. Condition much the same as in the original physical examination. Patient delirious, had been so since September 1st; Macewen present; very slight reaction of pupils to light; temperature, 105°; pulse, 110, slightly irregular; respiration slow. A lumbar puncture was done and 20 c.c. cloudy, yellow fluid was drawn. As no organisms had been demonstrated in previous fluids, 15 c.c. of antimeningitis serum was administered. An examination of this fluid showed the streptococcus pyogenes in smear and culture. It was accordingly recommended that anti-streptococcus serum be administered both intravenously—as streptococcus infections are likely to be generalized—and intraspinally. Also that streptococcus vaccine be given.

September 5th, lumbar puncture, about 40 c.c. of fluid withdrawn and about the same amount of antistreptococcus serum administered. This fluid was examined by us and also showed streptococcus pyogenes in smear and culture.

September 6th, 40 c.c. antistreptococcus serum given intravenously.

September 7th, 1,000,000,000 streptococus vaccine given—the large dose was due to a misunderstanding, but no bad results followed.

September 8th, 40 c.c. antistreptococcus serum given intravenously.

After September 5th some little difficulty seems to have been met by the interne in doing the lumbar puncture, so no more were done and we were not called to see the case, as he seemed to be improving under the treatment.

On August 31st, urotropin was begun—30 grains per day. This was increased three or four days later to 120 grains per day, and was continued until September 14th, when the urine began to show pus. The urotropin was then cut down to 30 grains per day until the temperature became normal, September 20th.

The patient left the hospital October 6th in very good condition, except for a slight tremulousness. About two weeks later some pain in the back developed and the patient returned to the hospital November 4th, complaining of pain in the back and along the sciatic nerves. Under the action of salicylates and counterirritants, the pain cleared up, but the impaired sensation and weakness existed for some time. The case was discharged improved in January. Neuritis following meningitis happens occasionally.

Recoveries from streptococcic meningitis are extremely rare. A careful search of the literature reveals but three.

Alexander[1] reports a case of chronic suppurative otitis media complicated by purulent labyrinthinitis and subdural abscess and pachymeningitis in which lumbar puncture revealed a purulent fluid with streptococcus in smear and culture. A radical operation, opening the vestibule and incision of the dura, was followed by recovery. Alexander claims that this is the first case of undoubted healed streptococcic meningitis.

A. Netter[2] reports a case of a child of seven with double otitis media complicating measles in July, 1900. Two punctures were done and the streptococci were cultivated from spinal fluid. The child recovered with no sequelæ. No medication was mentioned. Netter advises the use of lumbar puncture, warm baths and intravenous injections of colloidal silver.

Tedesco[3] reports a case of a girl twenty years old who was sick April 12, 1911. Headache, Kernig, strabismus, optic neuritis on both sides. A lumbar puncture April 16th showed pus and streptococci by culture. The pressure was greatly increased. April 19th, a second puncture showed a sterile fluid under less pressure. The patient went on to recovery.

Holger Mygind,[4] of Copenhagen, reports cure by operation of 6 cases of otitic meningitis, but does not state the nature of the infection. The gravity of streptococcus infections is generally recognized. Lermoyez, in 1908, states that while cases of otogenic meningitis due to staphylococcus and pneumococcus sometimes recover, after operation, streptococcus meningitis never does. Körner also claims that streptococcic meningitis is incurable. Of course, these claims are now controverted, but they serve to show how serious this infection is.

During the past four years we have seen 18 cases of streptococcic meningitis, of which only the one described above recovered. Five of these were due to streptococcus mucosus capsulatus—considered by most as belonging to the pneumococcus group, the rest to the streptococcus pyogenes group. In 2 of the cases the histories were lost. Of the remaining 16 there was no history obtainable bearing on the condition in 5 cases; 5 cases gave a previous history of middle ear disease or mastoid; 2, of scarlet fever, middle ear and mastoid; 1, of operation on the nose; 2, of trauma of the head; 1, of whooping-cough. Of these cases 7 received antistreptococcus serum, 5 receiving more than one dose intraspinally, intravenously or both. One of these recovered, as reported, and the other 4 showed considerable temporary improvement, though 3 were seen six days or more after the onset of symptoms. The fluids, too, showed improvement, the organisms becoming more and more intracellular and in 1 case diminishing in numbers.

The fourth case is deserving of a little special consideration. The child, H. W., eight years old, was convalescing from scarlet. Otitis media developed, and, on October 26, 1913, an operation for mastoid was performed on the right side. She was seen October 28th. A lumbar puncture had just been done by the interne and our examination had revealed streptococci, so 5 c.c. of serum was given intraspinally, 20 c.c. subcutaneously and 100,000,000 streptococcus vaccine was injected. On October 29th the condition was somewhat better—15 c.c. of very cloudy fluid was removed by lumbar puncture, 10 c.c. of serum was injected intraspinally and 20 c.c. subcutaneously. This fluid showed phagocytosis of the streptococci and a slight diminution in numbers.

On the 30th, the otologist in charge of the case decided to do a radical operation and try drainage through the skull and requested that the lumbar punctures be discontinued. On November 1st the child was very much worse and lumbar puncture was again requested; 20 c.c. of very cloudy fluid was withdrawn, 15 c.c. of serum was injected intraspinally and 20 c.c. subcutaneously, and 150,000,000 of vaccine was given. On November 2d and 3d lumbar puncture was performed and the serum was injected both intraspinally and subcutaneously. While some improvement was noted November 2d, it did not continue and the patient died on the 3d. It is to be observed in this case that the operative measures failed to give adequate drainage. While it is very probable that the patient would have died in any event, there was some improvement with the use of lumbar puncture and serum and vaccine therapy, both in the clinical condition and in the laboratory findings of the fluid. It is interesting to note that of the 4 cases of cure only 1 had undergone operation.

In Archives of Pediatrics, February, 1913, Dr. Irving S. Haynes, in the "Treatment of Meningitis by Drainage of the Cisterna Magna," urges strongly surgical interference in septic meningitis, even in epidemic meningitis, if improvement does not follow in twenty-four hours after the first administration of serum. In discussing the diagnosis of meningitis and the indications for operation, he says: "Kopetsky was the first to demonstrate that the disappearance of sugar from the cerebrospinal fluid was an invariable result of bacterial infection, that the absence of sugar could be determined before the bacteria themselves could be found and, further, that in conditions simulating meningitis, but not caused by bacterial infection and which did not eventually result in meningitis, the sugar did not disappear from the fluid."

With the statement that the sugar invariably disappears from the cerebrospinal fluid in bacterial infections and that its absence may be demonstrated before the presence of the bacteria we strongly disagree. In an examination of over 1,050 fluids we have never seen an instance in which the disappearance of sugar from the fluid preceded the appearance of organisms except in a few instances of apparently recovering epidemic meningitis where the organisms had disappeared from the fluid, but the sugar had not yet returned. On the contrary, in a fair proportion of fluids there is sugar present, though decreased in amount together with the organisms. For instance, of 88 known tubercular fluids 73 per cent. gave a good reduction, and of our last 117 specimens of purulent fluids 59 gave a reduction. We have observed that fluids that have for any reason not been examined within a few hours after withdrawal are much more likely to show the absence of sugar, and we think this may be a possible reason for the contrary findings of Kopetsky and Haynes. In view of these facts, we consider the presence or absence of sugar of prognostic not diagnostic value, as we have repeatedly observed that it is more likely to be present in favorable or recovering cases.

With the statement that in conditions simulating meningitis, but not caused by bacterial infections and not developing into meningitis, sugar does not disappear from the fluid, we agree in general. We have, however, had one fluid from a case of heat stroke that did not reduce Fehling's, though it was normal in other particulars. The child made an uneventful recovery.

Referring to the absence of sugar from the fluid as a very early sign, Dr. Haynes goes on to say: "Further, I urge that the same method of treatment (surgical) is demanded in the early cases of diplococcic infections as in any of the other varieties, because we do not know at this early hour which germ is present, and when we do know it may be too late to save our patients. The necessary conclusion is that surgical relief must be given in all cases of septic meningitis. Therefore, I maintain that if this first examination shows the loss of a proper reducing agent from the cerebrospinal fluid, but the actual germ is not detected at this examination, the case must be operated on at once. However, if at this first examination the diplococcus of Weichselbaum is detected, then the meningitis serum should be given, a delay of not more than twenty-four hours follow, and if there is no improvement then an operation should be done. Because a second injection of serum may not be entirely harmless, its probability of relieving the disease diminishes with the lapse of time and most serious of all such delay renders futile any surgical effort."

This method of treating ordinary epidemic meningitis we think quite unjustifiable. In cases of septic meningitis, the outcome of which is practically always fatal, it might be tried. It does not seem to us that the beneficial effects of surgical treatment have been demonstrated at all conclusively. Haynes goes on to say: "Unfortunately, all of my own cases have died. This is no discredit to the operation nor does this result invalidate my contentions. These results simply mean that operative relief must be offered at the beginning of the disease. Delay brings disaster."

We cannot see how the fact that late operative measures are unsuccessful proves in any way the value of early operative measures. We have had very little opportunity to try the effect of early lumbar puncture and serum therapy in cases of septic meningitis—but 2 of our own cases were seen before the sixth day. Of these, one recovered, the other was improving under lumbar puncture, but failed rapidly after operative measures were instituted. This operation was not, however, the one recommended by Haynes.

It is certain that meningitis due to any other pyogenic organism than the meningococcus is a very grave disease and the chances of recovery with any method of treatment are exceedingly small. But, as has been already pointed out, of the four known cures of streptococcic meningitis three were with lumbar puncture and one with operative measures.

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TREATMENT OF THE SITE OF VACCINATION WITH TINCTURE OF IODIN AND WITH 4 PER CENT. ALCOHOLIC SOLUTION OF PICRIC ACID

Frank S. Fielder and Samuel Parnass

During 1914 and 1915 several series of experiments were performed for the purpose of finding a practical and easy method of preparing the arm for vaccination and of lessening the severe local reactions and secondary infections that occasionally follow vaccination against smallpox.

I. Tincture of Iodin in Preparing the Site of Vaccination.

The tincture of iodin U. S. P. was tried in full strength, and in dilution with 95 per cent. alcohol to half strength and one-third strength; applying it to the skin and allowing it to dry prior to vaccination. As the table below shows, it materially impaired the potency of the vaccine virus.

		Insertions	Successful	% Successful	Quality and Size of Vesicles Obtained		Insertions	Successful	% Successful	Quality and Size of Vesicles
Tct. of iodin U. S. P. not washed off.	Full strength	10	1	10	poor	*Controls* Same virus; arm washed with alcohol. *No iodin*	50	50	100	good
	Half strength	10	5	50	fair		74	74	100	good
	⅓ strength	11	5	45.5	fair		49	49	100	good
Tct. iodin full strength washed off *thoroughly* with 95% alcohol.		47	47	100	good	*Controls* As above.	119	119	100	good
Tct. iodin full strength *partially* removed with alcohol		10	10	100	5 good and 5 fair size	*Controls* As above.	20	20	100	good

CONCLUSIONS

(1) If tincture of iodin in any efficient strength is applied, allowed to dry and not removed, vaccination through the iodized area will impair the potency of the vaccine virus. Many complete failures occur, and the vesicles obtained in the cases that do " take " are below par in quality and size.

(2) If it is applied, allowed to dry and then *thoroughly* washed off with alcohol until almost all the coloring of the iodin has been removed, no impairment of the vaccine follows and the results are uniformly satisfactory,

(3) If the iodin is carelessly or only partially removed with the alcohol, the vaccination takes, but the resulting vesicles are below the standard in size in half of the cases.

II. Tincture of Iodin Applied After Vaccination.

Albert & Alden[1] recommended the application of the tincture of iodin to the vaccination vesicle as soon as possible after it has formed (5 to 7 days after vaccinating), a second application two or three days later, and the repetition of same to be continued at intervals of two days until a dry crust resulted. *Result:* The applications of iodin either prevent pustule formation or so limit it that the resulting vesicles or pustules soon dessicate and form a small, dry scab. In not one of 116 cases so treated was there secondary infection with pus-producing bacteria, whereas about 30 per cent. of those not so treated were secondarily infected to a greater or less degree (though in many of these the pus formation, other than that of the pustules of vaccinia, was but slight).

We repeated the above experiment on April 27, 1915, making 54 insertions on previously unvaccinated children with virus No. 2243, and applying the tincture of iodin U. S. P., diluted with an equal part of 95 per cent. alcohol, to the vesicles and the surrounding skin for about ⅜ inch beyond each vesicle, at 5 days after vaccinating and every two days thereafter until 5 applications were made in all. On same date, April 27th, using same virus, 42 other insertions were made on previously unvaccinated children, and these latter cases were used as controls, making no after applications of iodin to them.

Result: The only effect that the applications of the iodin solution had was a diminution in the early local inflammatory reaction (areola) by about one-third. There was no difference noted between the iodin-treated cases and the controls in the drying of the vesicles or in the percentage of secondary infections.

III. Four Per Cent. Alcoholic Solution of Picric Acid Applied After Vaccination.

Schamburg & Kolmer,[2] after testing the comparative merits of picric acid and phenol as a local antiseptic, applied to the site of vaccination a 4 per cent. alcoholic solution of picric acid 48 hours after vaccinating and continued the application daily until the 14th day after vaccinating. They vaccinated 22 cases, 20 of which were successful and 2 failed; also vaccinated 9 other cases, 2 of which failed and the 7 successful ones were used as controls.

Results: Picric acid (4 per cent. alcoholic solution) is about four times as efficient as phenol as a local antiseptic, and the common organisms on the skin are lessened in number by its application.

Four per cent. alcoholic solution of picric acid applied to vaccination area 48 hours after insertion of lymph and repeated daily, does not interfere with the success of the vaccination.

It lessens the degree of the local inflammatory reaction.

Patients are not so apt to exhibit constitutional disturbances.

The epithelial covering of the vaccination lesion is hardened and there is decreased liability to extraneous bacterial infection. This is doubtless due in part to the antiseptic properties of the solution applied.

We used the 4 per cent. alcoholic solution of picric acid in the following experiments:

On March 3, 1915, 10 previously unvaccinated children were vaccinated with vaccine virus No. 2252, three scarifications—Nos. 1, 2 and 3—made on each child's arm. The arms were prepared by cleansing with plain alcohol, 95 per cent., which was allowed to evaporate before vaccinating. At two days after vaccination a 4 per cent. solution of picric acid in 95 per cent. alcohol was painted over scarifications Nos. 2 and 3 and over their surrounding skin about ⅜ inch beyond each scarification. Same solution was applied to the formed vesicles Nos. 2 and 3, at 3, 5, 7, 9, 10, 12 and 14 days after vaccinating. Vesicle No. 1 upon each case was used as control, no picric acid being applied.

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[1] Iowa Med. Jour., Apl., 1914, p. 180.

[2] Lancet, LXXXI, No. 4603, Nov. 18, 1911, p. 1397.

Observations: Each case was observed every other day until after the dried crusts had fallen off the child's arm, and the effects of the picric acid solution may be briefly stated as follows. Vesicles Nos. 2 and 3 were *hardened, dried and shrunken* at an earlier date than No. 1, and thus escaped being scratched and probably infected by child; the *local inflammatory reaction around vesicles Nos. 2 and 3 was strikingly less than that around vesicle No. 1*, there was hardly any inflamed area around the vesicles that were treated with the picric acid solution.

On April 13th, 23rd and 27th, 1915, 28 previously unvaccinated children were vaccinated with vaccine virus No. 2243, three scarifications made on each child's left arm. Forty-eight hours after vaccinating and daily thereafter for two weeks a solution of picric acid, 4 per cent. in 95 per cent. alcohol, was applied to each vesicle and the neighboring skin. At about the same time 14 other children were similarly vaccinated with same virus, but received no after applications of picric acid solution. The latter 14 cases served as controls. The results obtained in this number of cases were similar to those noted in the first 10 cases. In 21 of the 28 picric acid cases the areolae surrounding the vesicles and pustules were very faint, while in the other 7 cases the local inflammatory reaction was more marked, but still far less than that noted in an ordinary case of successful vaccination with efficient virus. The 14 controls showed the usual inflamed areolae about each vesicle and pustule.

Infection of vaccination vesicles: Total number of vesicles that were treated with the picric acid solution was 104. Total number of vesicles infected was 1. Percentage of infected vesicles 0.96 per cent.

Total number of vesicles to which the picric acid solution was not applied was 52. Total number of vesicles infected was 3. Percentage of infected vesicles 5.76 per cent.

Despite the evident shrinking and drying effects of the picric acid solution upon the vesicles, the resulting crusts did not fall off earlier than those vesicles to which the picric acid solution was not applied.

The resulting vaccination scars were about the same in the picric acid series as in the series of controls. In 8 of the first 10 cases, vaccinated March 3, 1915, the scars of vesicles Nos. 2 and 3 were the same as scar No. 1. In 6 of these 8 the scars were small, regular in outline, with even surfaces that were very slightly below the level of the surrounding skin. In one case there were definite pits left after the crusts Nos. 1, 2 and 3 had fallen off. In the eighth case there were deep excavations at the site of each take. In only two of the ten cases were scars Nos. 2 and 3 smaller and on a higher level than scar No. 1. In the lot of 42 cases vaccinated in April there was no appreciable difference between the scars of the 28 cases that received the picric acid treatement and those of the 14 cases that did not receive any after treatment.

General Conclusions.

1. Tincture of iodin may be used in preparing the site of vaccination, but it must be thoroughly removed with alcohol before the vaccine is applied, to avoid impairing the potency of the virus.

2. A 4 per cent. alcoholic solution of picric acid, applied two days after vaccination and continued every two days up to the end of the second week, does not interfere with the normal evolution of the vaccine vesicle. It has an excellent effect in lessening the early inflammatory reaction and in preventing secondary infection. It is superior to tincture of iodin in this respect and is so easy to apply that it may well be intrusted to any fairly intelligent mother.

THE UTILIZATION OF "REACTOR" MILK IN TUBERCULO-MEDICINE

Charles B. Fitzpatrick

(*Reprinted from the Proceedings of the Society for Experimental Biology and Medicine*, 1915, *xiii, pp.* 35-37)

We cannot in the study of tuberculosis get away from the disconcerting observation that it is the infected individual who is immune or possessed of increased resistance to tuberculosis. Our previous works[1] on immunity and tuberculosis led me to endeavor to ascertain to what extent milch cows, that gave no clinical signs of tuberculosis, and yet reacted to tuberculin, could be considered immune or possessed of increased resistance. Furthermore, to ascertain if the milk and serum of such "reactors" gave evidence of possessing antibodies or other healing bodies not contained in the ordinary milk and serum of cows not infected with tuberculosis. It is occasionally observed that if a cow which has reacted to tuberculin, be allowed to live, it thrives, apparently even better than some of the non-infected members of the herd. Ten of these "reactors" which were in especially prime condition were carefully selected because they thrived, and gave the physical evidence of having withstood the natural infection, in short because they appeared to be immune or the disease arrested. The milk when injected into guinea-pigs did not produce tuberculosis. The milk was also tested by the Bordet-Gengou phenomenon for tuberculosis and gave negative reactions. The blood-sera of nine were also examined for this reaction; four were definitely negative; four gave a weak reaction, and one a decided reaction but not strong enough for diagnosis. One of these cows went dry and the milk of another was excluded because it readily killed mice in comparatively small doses, when injected subcutaneously. The serum of these cows, when added to glycerin-bouillon cultures of the tubercle bacillus did not inhibit their growth. The ten autopsies on these "reactor" cows showed slight localized lesions in the lungs, and in the bronchial and posterior mediastinal glands in nine cows and in one cow slight generalized lesions were found.

Seven moderately advanced cases of adult pulmonary tuberculosis were fed daily a quart of this "reactor" milk over a period of three months. They gained an average of nine pounds. They increased this average gain during the next two months and ten days to 16 pounds. Six controls, *i. e.*, similar cases living under like conditions were given pasteurized milk, during the same period. They lost an average of four pounds. They increased this average loss during the next two months and ten days to six pounds. There was no noticeable alteration in the pulmonary conditions.

All the cases took three pints of milk a day. The special cases, one quart of "reactor" milk and one pint of pasteurized milk. The controls, three pints of pasteurized milk.

The "reactor" milk contained less butter fat than the pasteurized milk. The use of raw "reactor" milk, judging by its action upon two cases of adult pulmonary tuberculosis, is probably contraindicted in dysentery and hemorrhage. This dysentery case proved fatal. It was used in one far advanced case of adult pulmonary tuberculosis and apparently agreed with her, although her weight remained unchanged.

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[1] Proceedings of the Society for Experimental Biology and Medicine, 1910, VII, pp. 77-79; *ibid.*, pp 104-7; *ibid.*, VIII, pp. 24-28; *ibid.*, pp. 41-43; *ibid.*, Feb. 21, 1912, IX, pp. 49-51; *ibid.*, Feb. 19, 1913, Vol. X, No. 3, pp. 103-107; *ibid.*, Oct. 15, 1913; *ibid.*, June 6, 1914, Vol. XI, No. 6. Collected Studies 1913, Research Lab., Dept. of Health, N. Y. City, N. Y.

We mixed diphtheria antitoxine with milk for the purpose of determining by analogy if the tuberculous antibodies when present in milk, would be destroyed by pasteurization. These mixtures after having been heated were tested with toxin, in order to determine whether any destruction of antibody had taken place. We found that the antitoxine was not materially affected by heating at 60° C. for 20 minutes. Certain milks we have tested showed the presence of some natural substance or antibody which neutralized diphtheria toxin. I wish to thank Mr. E. J. Banzhaf for his aid in making these antitoxine tests.

THE COMPARATIVE IMPORTANCE OF PRESSURE AND OF TOXICITY OF TRIKRESOL IN SUBDURAL INJECTIONS OF SERA

Charles B. Fitzpatrick, James P. Atkinson and
Abraham Zingher

(*Reprinted from the Proceedings of the Society for Experimental Biology and Medicine,* 1914, *xi, pp.* 182-183)

As a result of several accidents reported as being due to intraspinal administration of antimeningococcus serum containing trikresol, a number of tests were made on dogs to determine if possible whether the fatal results were due to the influence of trikresol in the serum, to the serum per se, or whether they were due to pressure.

The records were taken with the kymograph, the pressure being taken at the carotid artery. The injections were made into the vertebral artery, the femoral artery and vein, the carotid artery and into the spinal canal.

The results which are somewhat contradictory, were as follows:

An antimeningococcus serum which had produced rashes and other disturbances in patients, caused well-marked depressions in 6.5 c.c. doses. Preservatives were chloroform and 0.4 per cent. trikresol. The injections were made into the femoral vein. A whole antipneumococcus serum preserved with chloroform tested in the same way produced death.[1]

Experiments (by F. and A.) with antimeningococcus serum to which was added varying quantities of trikresol (from .1 per cent. to .4 per cent.) gave no deleterious results when first mixed, but after standing one week in some cases depressions were obtained.

An injection of 2 c.c. of antimeningococcus serum prepared 19 days before the experiment by the addition of .4 per cent. trikresol was made into the vertebral artery. A marked depression resulted. Six c.c. of this serum also injected into the vertebral artery caused immediate clotting and were followed by a convulsion with almost complete cessation of respiration.

Further experiments carried out (by F., A. and Z.) on six dogs gave the following results:

1. As a rule, there was apparently no marked disturbance of blood pressure in normal dogs immediately after the lumbar subdural administration[2] by gravity or careful gentle pressure of moderate doses of antimeningococcus sera containing .3 per cent. trikresol.

2. Similar injections were made without marked disturbance in blood pressure of physiological saline solution, "*old*" antimeningococcus serum, antimeningococcus serum containing .3 per cent. and .4 per cent. trikresol, plain normal horse serum, normal horse serum containing chloroform, antistreptococcus serum containing chloroform and antipneumococcus serum containing chloroform.

3. Pressure appears to be a factor of real danger.

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[1] Vide, "Some Vaso-reacting Substances in Blood Serum," Proc. of Soc. for Exp. Biology and Medicine, 1912, LX, pp. 49-51.

[2] Lumbar puncture with skin incision through the skin and muscles was employed. Laminectomy was not used.

TISSUE EXTRACT AS A HEMOSTATIC
A PRELIMINARY REPORT

Alfred F. Hess

(*Reprint from Journal of American Medical Ass'n, April* 24, 1915. *Vol. LXIV, p.* 1395)

For some time we have been carrying out experiments with various preparations which increase the coagulability of the blood and would therefore be expected to be of value in stopping hemorrhage. The most useful of these preparations has been found to be one made from tissue. At first homologous tissue was used—in the case of bleeding in human beings, human uterine tissue; more recently, however, the liver or the brain of cattle has been employed. This tissue is obtained fresh from the slaughter-house, washed thoroughly free from blood, ground up in a machine, extracted in salt solution in the refrigerator and then filtered. In its preparation, aseptic precautions are observed as far as possible. The extract, which contains some fine suspension of tissue in addition to tissue juice, is made up with 0.3 per cent. of trikresol and preserved in this way. Aerobic and anaerobic cultures of this fluid have been found sterile. In addition to this liquid preparation, a powder has been prepared—the tissue has been desiccated and then finely ground up in a mortar.

Thromboplastic preparations treated with this small percentage of trikresol have been found to maintain their potency for at least a month. This is a distinct advantage. The difficulty has been that these preparations rapidly undergo autolysis with the formation of antithrombic substances, rendering them not only inert, but of negative value.

It is not intended in this brief report to enter into the details of our experiences with this product. We have found, however, in animals, that bleeding following a skin incision or incision into the liver is quickly checked by the local application of these preparations, and that when it has been given intravenously, for example, 2 c.c. of a 10 per cent. solution injected into the ear vein of a rabbit, it has been possible to shorten the coagulation time by one-half. In human beings we have had only a very limited experience with this thromboplastin given intravenously, having as yet confined ourselves mainly to its local use. Recently it rendered most welcome service when applied locally in two cases of hemophilia. One of these cases, a boy aged about 9 years, who has been under our observation for a year or more, was sent to the hospital on account of bleeding from the tongue which he had bitten while playing. As is so frequently the case in hemophilia, the bleeding was a mere oozing, which at first appeared to be of insignificant importance; it continued unchecked, however, in spite of the local application of fibrinogen, pure thrombin, calcium solution, gallic acid, serum and coagulose. The last-named preparation, as well as horse serum, was also injected subcutaneously, but without effect. After the boy had been bleeding almost continuously for four days, as he manifested increasing anemia and his pulse was becoming weak and his general condition critical, it was decided to obtain a donor and perform a transfusion on the following day. On the evening in question, however, as a last resort, a preparation of tissue extract was made from a uterus which had been removed in the operating room that very day. This was applied locally with almost instantaneous effect; the bleeding ceased and did not recur. In the test tube, the addition to blood of this tissue extract, which in the laboratory we have termed "thromboplastin," brings about coagulation with almost explosive rapidity. In the case of oxalated plasma (calcium must also be added), the clotting proceeds so quickly that no fibrin threads can be seen in the transparent clot. It is therefore to be expected that a substance with such properties would be especially indicated in hemophilia—a disease in which the primary, if not the entire defect, consists of a delay in the coagulation of the blood.

A second case bore out this supposition. This concerned a boy about 5 years of age, a marked bleeder with a pronounced family history of bleeding. The coagulation time of his blood had frequently been found to be remarkably delayed; ten drops at times took over three hours to coagulate in a small flat-bottomed tube. One day last December he began to bleed from a tear of the frenum of the tongue, and after various hemostatic measures had been resorted to in vain, he was referred to the Research Laboratory. There was found to be a soft clot incompletely covering the wound, from which there was slight but continuous oozing. On removal of this clot, the blood flowed more freely. Thromboplastin was applied on cotton for a few minutes, with the result that the bleeding ceased at once and did not recur.

These two cases seem to establish the value of thromboplastin in hemophilia. It remains to be ascertained whether it will be of value in purpura, a condition so frequently confused with hemophilia, but which is not characterized by a greatly delayed coagulation time, but by a marked decrease of platelets. It has been effective in controlling hemorrhage when the blood is normal, for example, after tonsillectomy, and therefore may be found of value in checking the bleeding which so frequently disturbs the operator in the surgery of the nose, brain, the abdominal organs, etc. When given intravenously, thromboplastin may prove to be of aid in checking various acute internal hemorrhages which are the result of ulceration or rupture of the vessel walls.

THE MOUTH-DRIP: A METHOD OF ADMINISTERING FLUID TO INFANTS IN GASTRO-ENTERITIS (ALIMENTARY INTOXICATION)

Alfred F. Hess

(*Reprinted from the Journal of the American Medical Association, February* 7, 1914. *Vol. LXII, p.* 452)

In the acute gastro-intestinal disturbances of infants, an excessive loss of fluid constitutes one of the greatest dangers to which the infants are subjected. Metabolism studies have shown this, and it is evident to even the casual observer. The dry skin, the parched mouth, the sunken eyes, the depressed fontanel, are striking evidences of a marked depletion of the body fluids. This condition is seen in its greatest intensity when the watery stools are marked and frequent and vomiting is repeated; but it is encountered also in those cases which are mainly toxic in character and which preeminently deserve Finkelstein's appellation of alimentary intoxication. In such cases there may be little or no vomiting, the diarrhea may have almost ceased, and yet the extreme prostration, the apathy or semistupor afford unmistakable evidence that the situation is critical. At this time, we now know, food must be at first entirely withheld, and then given only in minimal amounts. If it is given even in moderate amount and in correct formula it acts as a food-poison, increasing the stupor and leading rapidly to death. At this period of the disease the most essential therapeutic measure is to supply the infant, in one way or another, with a large amount of fluid. Almost all text-books emphasize this point, urging that under such conditions small quantities of water be given frequently. This can be accomplished in various ways. The fluid may be proffered every fifteen minutes or half hour by mouth, it may be introduced by means of hypodermoclysis, or, finally, it may be instilled into the rectum.

In the course of the past year I have made use of an expedient which seems to me serviceable in this connection, and therefore of sufficient importance to bring to the attention of those who have to treat this type of disease. In brief, it consists of applying what is popularly known as the Murphy drip-method (by means of which water is given drop by drop by rectum) to the giving of water by mouth—or, in other words, a "mouth-drip." This method has the advantage that fluid entering the upper intestinal tract is absorbed more completely than that which is given subcutaneously or by rectum. Furthermore, in acute enteritis the irritability of the bowel is so marked that the rubber nozzle is not well retained and, indeed, intensifies the diarrhea.

The apparatus (as shown in the accompanying illustration) is the same as the rectal drip except that, instead of a hard rubber nozzle, an ordinary rubber nipple is attached to the end of the tubing by means of a glass connecting-piece. The baby is encouraged to suck on this nipple for the greater part of the day and in this way obtains a large amount of water, hypotonic salt solution, Ringer's solution or other fluid.[1] I have had the drip so regulated by means of a pinch-cock that from about 25 to 30 drops per minute fall from the glass dropper into the tube. In every case of enteritis in the hospital an apparatus of this kind has been suspended next to the bed. By this means these infants have received about a quart of water in the course of the twenty-four hours, the amount of fluid naturally depending on their thirst and on the degree of the poisoning. Often in the early stages, when the tongue is dry and parched, they will suck almost continuously on the nipple and cry when it is removed, whereas later, when entering on convalescence, they will no longer take to it so greedily. The nipple at times falls from the mouth, the infant releasing its hold. In order that the bed-covering shall not be made wet in this way a little saucer or plate is placed next to the baby's head. The nurse, therefore, has to replace the nipple from time to time. This does not entail near the amount of labor or individual attention which would be required were the infant to receive the same amount of fluid by bottle or spoon in the course of twenty-four hours. The accomplish this end, the nurse would have to offer the water or other fluid many times in the course of an hour. In my experience, this is practically impossible in an infants' ward. If there is one nurse to one or two babies, she can devote her attention to supplying the infants with sufficient fluid; but in the ordinary infants' wards, so many other duties fall on the nurses that the infants, unable as they are to give

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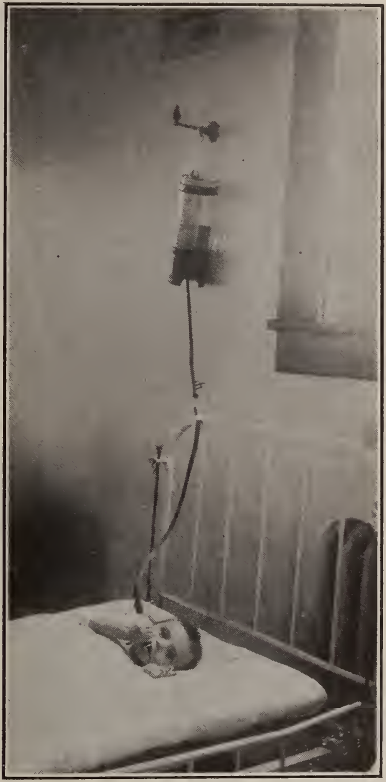
[1] The addition of a small amount of saccharin to the water frequently results in the babies' taking still larger quantities.



Mouth-drip. Infant sucking on nipple. The glass dropper and regulating stop-cock may be seen just above upper rail of bed.

Care should be taken that the tube does not drag, and, by its own weight, tend to be pulled away from the mouth of the infant. In order to avoid this, it can be suspended vertically (as shown in the illustration) or, still better, it can be so arranged that the part near the nipple rests horizontally on the bed. To obviate, furthermore, a tendency of the nipple to be pulled away, it will be noticed that the comparatively heavy glass dropper is placed at a considerable distance from the nipple. Another detail, perhaps worthy of mention, is that before the drip is started the fluid is allowed to fill the entire tube; after it is filled, the regulating pinch-cock is applied so that the fluid falls drop by drop.

utterance to their wants, lie often for hours in urgent need of fluid. It must, I think, be a common experience that when a spoonful of water is offered to an infant suffering from alimentary intoxication, it is gulped down greedily by the thirsty baby as if it had not received any water for hours. So great is the absorptive power of the mucous membranes in these cases that the tongue becomes parched in a very few minutes after water has been administered.

It may be urged that by keeping a nipple in the baby's mouth we are encouraging the formation of a bad habit. This criticism, however, can hardly be entertained in a consideration of the treatment of a disease involving a mortality so high as that of gastro-enteritis of infants. In the light of recent studies in metabolism, demonstrating the marked difference between the expenditure of energy in infants that are quiet and in those that are restless, it is, indeed, questionable whether this procedure does not possess additional advantages through its quieting effect and through the conservation of calories which it in this way accomplishes.

This " mouth-drip " is brought forward not with the idea of presenting a novel device, but merely as a simple method of facilitating the introduction of large quantities of fluid, more especially in alimentary intoxication, but also in other toxic conditions of infants and in pyelitis, in which large quantities of fluids are indicated. It is particularly applicable to the hospital ward, where the infants cannot have the individual attention of a nurse.

FULLERS' EARTH IN THE INTESTINAL DISORDERS OF INFANTS

ALFRED F. HESS

(*Reprinted from The Journal of The American Medical Association, January* 8, 1916, *Vol. LXVI, pp.* 106-107)

Of late the use of kaolin has been recommended for the treatment of a variety of disorders.[1] It has been reported to have therapeutic value in enteritis, in furunculosis and in vaginitis, and, more recently, has been recommended for application to the nose and throat in infections of these organs.[2]

The underlying basis of this type of therapy—the use of nonabsorbable substances—is not thoroughly understood; some believe that its value is due to the inhibition of the growth of bacteria, and others that, because of its adsorptive property, it possesses antitoxic value. Influenced by these favorable reports I prescribed kaolin in the intestinal disorders of infants. For this purpose it was either mixed with the milk or was given by the teaspoon. The results in either case were disappointing. In normal infants, kaolin occasioned only a slight degree of constipation, and in those manifesting diarrhea it was found not to reduce the number of stools materially. Dissatisfied with the results of kaolin I turned to the use of fullers' earth. Although these two substances are considered synonymous in the United States Dispensatory and the National Dispensatory they are by no means alike, either in their composition or physiologic action. It is difficult to give a standard analysis of fullers' earth, as it differs considerably, depending on the location in which it is obtained. However, the following analysis of this earth, as well as that of kaolin, may be regarded as typical:

	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Alkalies	Water
Kaolin	45.40	37.34	1.92	0.44	0.20	0.52	14.0
Fullers' earth	57.26	18.33	1.87	2.58	1.06	....	18.4

It will be seen that there is a considerable difference between the two substances, the main distinction being that kaolin has a far greater amount of hydrous aluminum silicate, and fullers' earth considerably more calcium. They are different, however, not only in their chemical composition, but also in their physical properties. One of the most noteworthy characteristics of these earths is their power to absorb various alkaloids. In view of this fact, they have been recommended[3] as antidotes in the case of various alkaloidal poisonings. In this respect fullers' earth stands preeminent, as it is characterized by its porosity and, as shown by Parsons[4] "carries an unusually large proportion of colloids of high adsorptive power." In this quality various specimens of earth are by no means uniform. This adsorptive property can most readily be tested by determining the quantity of earth required to remove from solution a given amount of dye. In making tests of this kind, we followed the procedure reecntly recommended by Fantus,[3] in which a 1 per cent. malachite green and 0.25 per cent. morphin solution are employed. It seems hardly necessary in a paper presenting a clinical view to give the data of these various examinations. The results may be summarized by the statement that fullers' earth possessed a greater degree of adsorptive power than kaolin; but that among the six specimens of earth which were obtained from various parts of the United States, there were marked individual variations.

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[1] Hektoen, L., and Rappaport, B.; The Use of Kaolin to Remove Bacteria from the Throat and Nose, The Journal A. M. A., June 12, 1915, p. 1985.

[2] In three diphtheria carriers in which kaolin was applied to the pharynx in order to rid it of bacilli, it seemed to exert no effect, although it was used many times a day for several days.

[3] Fantus, Bernard: Fullers' Earth: Its Adsorptive Power, and Its Antidotal Value for Alkaloids, The Journal A. M. A., May 29, 1915, p. 1838.

[4] Parsons, C. L.: Mineral Technology, Bull. 71, Bureau of Mines, Dept. of the Interior, October, 1913.

It was realized, however, that the value of these earths in the intestinal tract cannot be determined by their ability to adsorb dyestuffs, but must be learned by direct clinical observation and experience. Accordingly, fullers' earth was given to a considerable number of normal infants, in order to test its physiologic effect; to this end, 1 ounce of the earth was given in the day's feeding. This mixture was taken without difficulty by the infants, and was found to occasion no disorder whatsoever. Its sole effect, which may be stated to have been constant, was that it induced constipation. The stools became firm, dry and formed; indeed, in some cases the constipation became so marked that the addition of the earth had to be discontinued. The preparation was then given to infants suffering from indigestion, as manifested by diarrhea, accompanied in some instances by vomiting. In these cases, the earth was either added to the food, consisting of the diluted milk, or it was given by teaspoon every hour or two. Either or both of these methods can be used. No difficulty was experienced in giving the powder suspended in a little water, especially when it was sweetened by means of saccharin (two-fifths grain to 1 ounce of fullers' earth). In severe cases of enteritis, no food whatsoever was given, but merely teaspoonful doses of this preparation as often as every half hour. In some cases it was fed through the stomach tube—1 or 2 tablespoons being introduced in this way three times a day.[5]

As in every type of therapy, success is not invariable. We may state, however, that this therapeutic agent has had a greater effect on inhibiting the diarrhea than has bismuth, chalk mixture or other drugs which are commonly used for this purpose. In some cases it has also seemed to exert a sedative effect on the stomach, as judged by the fact that vomiting ceased in the course of this treatment. In no instance were any harmful effects noted, although the earth was given to patients suffering not only from enteritis, but also from pneumonia and nephritis.

When we turn to consider the modus operandi of this treatment, we tread on ground that is by no means firm. We know that this earth, as Fantus has shown, is "capable of lessening in the human the absorption of substances that are readily absorbed." It is therefore possible that this property is of value in its therapeutic application—that it adsorbs toxins and bacteria, which would otherwise be harmful for the body. In this connection we may add that experiments carried out with solutions of dye showed that casein likewise possesses adsorptive properties to a considerable extent. This naturally raises the question whether casein does not exert an influence on the intestinal tract and its contents owing to its physical as well as to its chemical composition, and whether we are not giving too little thought and credit to this point of view in our dietetic treatment of diseases. It may well be, for example, that part of the therapeutic success which is so regularly obtained by the use of albumin milk (casein or protein milk), is the result, not only of the antifermentative action of the casein, but of its adsorptive properties as well. However this may prove to be, I wish to point out the difference in therapeutic efficiency between kaolin and fullers' earth, and also to recommend the use of the latter in the gastro-intestinal disorders which are so common in early infancy.

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[5] Lloyd's reagent, a preparation composed of very fine particles of fullers' earth, was given to a number of infants, but did not seem to possess any advantages over the inexpensive common specimens of fullers' earth.

A PROTECTIVE THERAPY FOR MUMPS *

ALFRED F. HESS

(*Reprinted from the American Journal of Diseases. of Children, August,* 1915. *Vol. X, pp.* 99-103)

The protection afforded to a man against epidemic diseases through the employment of prophylactic antiserum injection is of course best illustrated by the case of diptheria. It is likewise known that many infections to which animals are subject can be prevented by a similar form of passive immunization. While the number of experimental infections which can be prevented in this manner is not large, it is a notable fact that the blood of animals which have recovered from hog cholera carries a protective principle capable of warding off, for a time, infection with the filterable virus of that disease.

That human blood of one individual can be injected safely into another individual is of course a commonplace. Recent experience with the transfusion of large quantities of blood is convincing in establishing the value and safety of this procedure. Experience has also shown that human blood carrying immunity principles can be employed as therapeutic agents in several infectious diseases in man. Thus McKenzie and Martin[1] employed the blood serum taken from patients who had recovered from epidemic meningitis in the treatment, by intraspinal injection, of similar cases of meningitis. The blood taken from patients who have recovered from scarlet fever has also been employed,[2] and apparently with advantage, in the treatment of severe toxic cases of scarlet fever. Flexner and Lewis[3] have shown that the blood serum derived from monkeys or human beings who have recovered from epidemic poliomyelitis is capable of preventing in monkeys the paralysis which follows the intracerebral injection of the poliomyelitis virus, and Netter[4] has employed the serum taken from recovered human beings for the treatment of patients with acute poliomyelitis by intraspinal injection.

It is obvious, therefore, that the employment of the blood of one individual as a therapeutic agent for another is a method which in itself is without danger, and, under certain circumstances, offers advantages to the patient to be secured in no other way. Such small objections to the employment of blood as a therapeutic agent as exist can be obviated by the selection of suitable donors.

In view of these considerations it is interesting to consider what may be accomplished in preventing mumps, or epidemic parotitis, especially in institutions in which a large number of cases arise from time to time. Mumps is still to be ranked among the highly contagious infective diseases of unknown etiology, the specific causative microbic agent not having been discovered. The disease is, however, one in which one attack yields protection that is general and persistent. The question arises, therefore, whether the blood of persons who have recovered from one attack of the disease does not contain immunizing and protective principles which can

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* Presented before the Pediatric Section of the Academy of Medicine, April 8, 1915.

[1] McKenzie and Martin: Brit. Med. Jour., Oct. 31, 1908, p. 1341.

[2] Reiss and Jungman: Deutsch. Arch. f. klin. Med., 1912, cvi, Nos. 1 and 2. Reiss: Therap. Monatsh., 1913, xxvii. No. 6.

[3] Flexner, S., and Lewis, P. A.: Experimental Poliomyelitis in Monkeys, Jour. Am. Med. Assn., Aug. 20, 1910, p. 662.

[4] Netter, F.: Bull. de l'Acad. d. méd., 1914, lxxviii, 523.

TABLE 1.

DATA OF DONORS AND OF INJECTED PATIENTS.

Donor	Age	Date of Mumps	Donee	Age	Date of Inoculation	No. of c. c. Injected
1. S. C.	5½	1/30/15	H. S.	4	2/1/15	6
2. P. F.	4	1/30/15	B. K.	4	2/1/15	6
3. G. D.	4	1/31/15	S. G.	4	2/1/15	8
4. A. S.	5⅓	1/29/15	P. K.	3	2/1/15	6
5. I. R.	4	1/22/15	Y. G.	3½	2/1/15	6
6. J. S.	4	1/22/15	F. G.	3½	2/1/15	8
7. R.	6⅓	1/22/15	E. Z.	2½	2/2/15	7
8. H. H.	5	1/22/15	R. G.	2½	2/2/15	8
9. S. G.	5⅓	1/22/15	M. H.	4	2/2/15	6
10. G.	5	1/23/15	D. E.	3¼	2/2/15	6
11. M.	2½	1/23/15	S. S.	2¼	2/2/15	8
12. R.	4½	1/23/15	H. L.	2	2/4/15	6
13. K.	3½	1/24/15	D. G.	2	2/4/15	6
14. S.	3½	1/25/15	H. I.	3	2/4/15	6
15. M. G.	4	4/ /14	B. Z.	2¼	2/4/15	6
16. D. G.	5	4/ /14	I. S.	1½	2/4/15	8
17. L. S.	5	4/ /14	H. C.	1⅔	2/4/15	6
18. B. K.	6½	3/ /13	S. G.	5½	2/4/15	6
19. J. S.	5½	12/ /12	L. S.	4½	2/4/15	8
20. L. S.	2⅓	12/ /12	L. S.	5½	2/4/15	6

be employed in the protection of other exposed persons. Since the collection and the injection of the blood, as indicated above, are readily carried out, an effort was made this winter to control by this means an epidemic of mumps prevailing in the Hebrew Infant Asylum, which for the second time in two years was visited by a severe epidemic. The epidemic was well under way when we determined to undertake this prophylactic therapy, as may be seen from Table 2, which shows the cases as they developed in the various wards, both previous and subsequent to the injections. At the time treatment was begun, about forty cases of mumps had broken out in the institution within the past month, and, as Table 2 shows, new cases were developing almost daily. The conditions surrounding an institution of this kind are peculiarly favorable for judging the effect of prophylactic treatment of the various infectious diseases, as most of the children are admitted during infancy, and we have complete knowledge of the infectious diseases which they have contracted while under our care.

Twenty children were given protective injections. They were, for the most part, children who had entered the institution before the age of one year. Naturally, none were injected who had had mumps in the course of the epidemic of the winter of 1912-13 and who had acquired immunity in this way. The ages of these children, as well as of the donors, and the amount of blood injected, may be seen in Table 1. Whole blood was used and was injected at once intramuscularly.[5] The donors form three groups: The first includes children, four in number, who were just recovering from mumps; the second comprises ten children who had recovered from the disease about ten days previously; there is a third group, composed of six children who had had mumps one or two years before. It should be added that the results of the treatment in the last group cannot be considered convincing, in that three (15, 16 and 17) must be entirely left out of consideration, as they did not happen to come in contact with active cases of the disease, and because the other three (18, 19 and 20) had been in the institution but six weeks, so that we did not possess first-hand knowledge as to their susceptibility to mumps. As was to be expected from the experience of others, there were no disagreeable manifestations following the injections—neither a rise of temperature nor local reaction. The epidemic was so widespread that only three of the children under treatment did not come into contact with the disease. There are therefore seventeen who must be regarded as having been not only susceptible, but also exposed to mumps. It is therefore striking to note that among these seventeen, none contracted the disease. That the injections were effective in bringing about this result may be seen from a glance at Table 3, which shows that in the wards where the children were not protected by injection, fully one-third, or even one-half, came down with the disease.

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[5] The blood was aspirated in the usual way and immediately injected into the patient. The procedure required so short a time that there was no need of adding any anticoagulant, and there was no disturbance due to premature clotting.

TABLE 2.

Occurrence of Mumps in Wards Before and After Injections.

Date	Ward No.				
	4	6	10	11	12
Jan. 22	1	6	1	1	0
Jan. 24	0	0	0	0	1
Jan. 25	1	0	0	0	0
Jan. 26	2	3	0	0	0
Jan. 28	0	0	0	0	1
Jan. 29	0	0	1	0	0
Jan. 30	2	1	0	0	1
Jan. 31	0	1	0	2	1
Feb. 8	0	1	1	1	0
Feb. 10	0	0	1	1	0
Feb. 11	1	0	0	2	4
Feb. 14	2	1	0	1	4
Feb. 15	3	0	0	0	1
Feb. 17	2	0	0	0	1
Feb. 20	3	0	0	0	0
Feb. 22	2	0	2	1	0
Feb. 23	0	0	0	1	0
Feb. 25	0	1	3	1	0
Mar. 2	0	0	0	2	2
Mar. 3	0	1	0	1	1
Mar. 4	1	0	0	0	0
Mar. 7	0	0	0	0	0
Mar. 18	0	0	0	0	0

Horizontal lines indicate period when injections were begun in the different wards.

Our results hardly seem to require extended comment. We may add, however, that by means of these injections we were able, in a large measure, to check the epidemic. It would seem that preventive treatment of this kind could well be carried out in institutions for children, and that this simple procedure might likewise be resorted to in the home where one or more children are exposed to infection. Our experience seems to indicate that the blood may, with advantage, be obtained from the donor even before the parotid swelling has disappeared, and that it possesses protective principles at this early stage. As is well known, the incubation period of mumps is long, about eighteen days, so that it is possible to resort to protective injection some days following exposure. It is naturally impossible to state the duration of immunity acquired in this way; but it seems reasonable to believe that it well outlasts the usual danger period of infection. This type of therapy probably can be resorted to in connection with epidemics of other infectious diseases, for example, measles, in which one attack confers a marked immunity.

TABLE 3

INCIDENCE OF MUMPS AMONG INJECTED AND NONINJECTED CHILDREN.

Ward No.	Total Census	No. of Susceptibles	Inoculated Cases	Date Transferred to Ward	No. Cases Developing Mumps Subsequently		Remarks
					Not Inoculated	Inoculated	
4	25	22	3 (Nos. 1, 12, 14)	2/15/15	11	0	Nine cases of mumps removed from this within previous three weeks.
6	30	19	1 (No. 13)	2/11/15	8	0	Twelve cases removed.
10	30	27	1 (No. 20)	2/15/15	5	0	Four cases were removed from this ward within previous three weeks.
11	34	32	2 (Nos. 18, 19)	2/15/15	7	0	Six cases were removed within previous three weeks.
12	34	34	10 (Nos. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11)	2/12/15	13	0	Ten cases were removed within previous three weeks.

THE USE OF A SERIES OF VACCINES IN THE PROPHYLAXIS AND TREATMENT OF AN EPIDEMIC OF PERTUSSIS *

Alfred F. Hess

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Last December pertussis broke out in the main building of the Hebrew Infant Asylum, an institution which houses about 375 children, ranging in age from a few days to 6 years. It soon became evident that the disease was destined to assume considerable proportions, and that we were on the threshold of an epidemic. An occurrence of this nature offered an exceptional opportunity to make an extended test of the value of vaccination in this disease.

The study of an epidemic within the confines of an institution has marked advantages over the study of an equal number of cases selected from hospital or private practice. In the first place, it must be evident that the fact that an epidemic originates from a single source, that the cases are due, in all probability, to infection with the same type of micro-organism, greatly simplifies the solution of the problem; it negatives the question of whether a difference in the severity of symptoms is due to a variation in virulence of the invading bacteria. In hospital or in private practice, on the other hand, the cases must always have originated from manifold sources. It is probably also of advantage, from the standpoint of comparison, that these institutional children belong to the same stratum of society, that they have for the most part been reared for a considerable period within the same walls, having the same daily routine, including similar food and an equal amount of outdoor life. These are some of the conditions which are insisted on in considering the course of experimental infection among laboratory animals, but which can rarely be controlled in a study of infection in man.

Many of the hospital studies of the treatment of pertussis are, however, open to the severer criticism, that owing to the poorly defined onset of this disease, there is no means of knowing how long the children were ill in their homes. This fact is naturally of great importance in considering the success of treatment. All these objections can likewise be raised in regard to cases treated in the dispensary or in ambulatory practice; in addition, this type of case is open to the criticism that one must rely solely on the opinion of ignorant parents in judging the efficacy of the treatment.

Prophylactic Use of Vaccine

We shall first consider pertussis vaccine in its prophylactic use. Here a field of exceptional promise presented itself. Two hundred and forty-four patients were inoculated with four different vaccines. Vaccine 1 consisted of a typical strain of the Bordet-Gengou bacillus obtained from Parke, Davis & Co., and two atypical strains. Vaccine 2 was " Bacterin " (Parke, Davis & Co.), which is stated to be composed of a pure culture of the bacillus of pertussis. Vaccine 3 was prepared from four typical cultures of the Bordet-Gengou bacillus isolated by Dr. Anna Williams of this laboratory. Vaccine 5 may be termed an autogenous vaccine in the sense that it was composed of three typical Bordet-Gengou strains isolated from cases included in this epidemic. Table 1 shows the results of the vaccinations. It may be summarized by the statement that *twenty of the 244 cases developed pertussis in spite of the prophylactic inoculations.*

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* Read before the Section on Diseases of Children at the Sixty-fourth Annual Session of the American Medical Association, Atlantic City, N. J., June, 1914.

TABLE 1

Prophylactic Vaccinations (244 Cases)

Vaccine	No. of Inoculations	No. Treated	Pertussis
1	2	35	10
2	3	10	1
3	3	141	6
5	3	58	3
		244	20

Let us examine this table somewhat more closely. It will be seen that Vaccine 1 shows by far the greatest number of failures. Two injections of the vaccine were given in each instance, one of 100 millions, the other of 200 million bacteria. Of the ten cases which developed pertussis, five showed the first symptoms two or three weeks after the first inoculation, one after twenty-five days, one after six weeks, two after an interval of two months or more. In considering the results of prophylactic inoculations, all cases were discarded and not included in the tabulation (Table 2) which shows the development of symptoms of pertussis within two weeks of inoculation.

TABLE 2

Details of the Twenty Cases Developing Pertussis Notwithstanding Prophylactic Vaccination

Name	Date Vaccinated	Type of Vaccine	Date of Onset of Pertussis	Severity of Disease
D. F.	1/12	1	3/ 5	+++
A. A.	1/12	1	1/30	+++
L. W.	1/12	1	1/26	+
B. A.	1/12	1	1/26	+
*A. S.	1/13	1	3/14	+++
N. K.	1/13	1	1/29	+
M. G.	1/13	1	3/14	+
I. W.	1/ 8	1	2/ 2	+
L. R.	1/13	1	1/29	+++
D. D.	1/13	1	4/ 1	++
A. H.	2/20	3	3/10	++
B. B.	4/17	3	5/ 6	+++
Y. G.	4/17	3	5/20	++
G. H.	3/24	3	5/ 8	++
R. T.	4/17	3	5/13	++
*A. S.	2/23	3	3/14	+++
J. S.	3/24	3	6/ 1	++
H. A.	4/14	5	5/24	+
J. B.	4/14	5	5/ 5	++
L. C.	4/14	5	5/12	+

*Received two varieties of vaccine.

When we turn to Vaccine 2 we find rather too small a number of cases to allow of a satisfactory estimate of its worth. Inoculations of 100, 200 and 400 millions were made at three-day intervals. The one case which developed pertussis showed symptoms two and one-half weeks after the first inoculation. It should be mentioned in this connection that five of the cases of this group developed ring-worm and were therefore sent away from the institution. As a result, the period of exposure to infection by pertussis was shortened in these cases.

The results obtained with Vaccine 3 are particularly interesting, as they are so much better than those obtained with the other vaccine. The same doses were given as of Vaccine 2, and with the same intervals. In twenty-four of this group of 141 cases, large doses were given by mistake, namely, 500, 1,000, 2,000 millions. One of these children nevertheless developed pertussis. This case (A. S., Table 2) is of especial interest in that the child received two prophylactic doses of Vaccine 1 about the middle of January, and three very large doses of Vaccine 3 towards the end of February, and notwithstanding this treatment came down with pertussis on March 14; the disease was exceptionally severe, whooping being continued for a period of ten weeks. The other cases, five in all, developed pertussis three weeks, six weeks, one month, and one about two months following inoculation.

As the result of prophylactic vaccination with these three vaccines it was determined to make a vaccine from cultures obtained from cases in our wards. Dr. Anna Williams was kind enough to prepare a vaccine of this nature as well as to make cultures from the throats of many of the children. It should be emphasized that the typical Bordet-Gengou organisms could be isolated in only five cases out of the thirty cultures taken. In most instances other hemophilic bacilli, frequently the influenza bacillus, were isolated. It may therefore be maintained with some justice that in our cases the symptoms may not in all instances have been incited by the Bordet-Gengou bacillus, and that this accounts for the failures in the use of Vaccine 5. It will be noted that of the 58 cases inoculated with this vaccine three developed pertussis. All these children received 100, 200 and 400 million bacteria. Two of the number began to whoop about one month after vaccination, and one three weeks following the initial injection.

One hundred and thirty children did not receive prophylactic inoculations. This group included a ward of twenty-five infants under the age of 1 year, and another ward of about thirty infants between the ages of 12 and 18 months. These children must be considered separately, as they were at all times in bed, so that the danger of contact infection naturally was far less than in the case of runabout children. Moreover, in the one ward comprising thirty infants, no case of pertussis developed, so that they were not subjected to infection and therefore should not be included among those exposed to pertussis. Only one case of pertussis occurred among the twenty-five infants under 1 year of age. That this case did not lead to further infection shows the difficulties in judging of the results of the prophylactic treatment. However, the fact that these infants did not come into close contact, that they were at all times in bed, should of itself exclude them from consideration. It would be a palpable error to compare infection in an infants' ward with that occurring in a ward of older children. This distinction should always be borne in mind in considering the degree of infection in institutions for children. If therefore we subtract these fifty-five cases—the thirty where no infection occurred and where consequently no exposure existed, and the twenty-five where exposure was greatly limited by absolute confinement to bed—we have eighty children who were not vaccinated. Of these eighty, fifty-nine developed pertussis. It will be remembered that of the 244 vaccinated cases, twenty came down with pertussis. There were five cases which cannot be included in the group of either the vaccinated or of the unvaccinated, as they began to whoop within a fortnight of vaccination; the case in the infants' ward constitutes a sixth case which cannot be considered.

It is difficult to determine the degree of exposure of the various children. No doubt it is true that at the very beginning of the epidemic, isolation was less complete and exposure to infection accordingly greater. This period, the early days of the epidemic, corresponds to the time preceding our employment of vaccine. However, granting a certain increased exposure of the unvaccinated at this period, we feel that the disparity in the ratio between the infected and the non-infected is so marked among those vaccinated and those unvaccinated—among the former twenty out of 244, or one in twelve, among the latter fifty-nine out of eighty or almost three out of four—that vaccination must have played a role. It should be mentioned in this connection that with the exception of the infants' ward, pertussis occurred in every ward in the building, and that quarantine regulations were enforced in the cases of children with suspicious cough or actually manifesting symptoms of the disease.

Table 3 shows the development of the cases both according to wards and according to months. It will be noted that in April the number of cases began to diminish. This time corresponds to the period of vaccination. In brief, vaccination was undertaken as follows: The 35 cases were inoculated with Vaccine 1 about the middle of January, and the ten cases with Vaccine 2 about the middle of February. At the very end of March and the beginning of April the large group of 135 cases were treated with Vaccine 3. In other words, prophylactic treatment on a large scale did not exist until the middle or end of April. This date corresponds with the beginning of the cessation of the epidemic. The epidemic may have been waning of its own accord; however, at this time, there was no abatement of pertussis

TABLE 3

Distribution of Epidemic According to Wards and Months

Ward	Dec.	Jan.	Feb.	March	April	May	June	Total
I	..	..	..	..	1	..	..	1
II	..	..	..	..	..	..	..	0
III	..	2	3	3	2	..	..	10
IV	*1	14	4	3	2	1	..	25
V	1	2	6	3	..	1	..	13
VI	..	..	..	1	..	..	..	1
VII	..	..	..	..	3	2	..	5
VIII	..	..	..	..	..	1	1	2
X	1	2	..	1	..	..	..	4
XI	1	3	1	5	..	..	..	10
XII	..	4	2	1	1	..	..	8
XIII	..	2	1	2	1	..	..	6
Total	4	29	17	19	10	5	1	85

*First case.

throughout the city. We are therefore of the opinion that the vaccine was of value in preventing infection and in limiting this epidemic, but that it cannot be relied on in each individual case as a prophylactic measure. It would seem probable that there is considerable difference in the potency of various vaccines, and, following the deductions to be drawn from our results, that a polyvalent vaccine of typical Bordet-Gengou bacilli is most efficacious.

Active Treatment

Vaccines were also used in this epidemic in the active treatment of pertussis. As in prophylaxis, four vaccines were employed; however, one of these (No. 2) was not the "Bacterin" mentioned above but the "combined vaccine" of Parke, Davis & Co., which is composed of the Bordet-Gengou bacillus, influenza bacillus, various hemophilic bacilli, streptococci, etc. Of the eighty-five cases of pertussis constituting the epidemic, sixty-five were treated by means of vaccines, and twenty were not vaccinated. No other treatment whatsoever was given to either group of patients. About an equal number of children were treated with each of the four varieties of vaccine; the cases were not selected, a change in the type of the vaccine being instituted whenever about fifteen children had been vaccinated. In some cases two vaccines were made use of just as with the prophylactic treatment.

Table 4 seems of especial interest in judging of the efficacy of the vaccine treatment. This table, it will be noted, is composed of the twenty severest cases of the epidemic. In compiling this list, we had recourse to the ward charts which showed the number of paroxysms of coughing and attacks of vomiting which each child had by day and by night, and were guided also by the opinion of the resident physician and of the head nurses. After the twenty cases had been selected in this way, a table was constructed to demonstrate their relationship to vaccination. It will be seen that of the total number only two received no vaccine treatment whatsoever. Five received both prophylactic inoculations and active treatment during the course of the disease; five were inoculated with two varieties of vaccine. This list, which is essentially a list of therapeutic failures, contains representatives of all four vaccines, but it will be noted that, as in the case of prophylactic vac-

TABLE 4

Vaccination in Relation to the Twenty Severest Cases

Case	Vaccine Prophylactic	Vaccine Curative	Case	Vaccine Prophylactic	Vaccine Curative
1	0	0	11	0	1
2	0	2	12	1	1
3	0	1 and 2	13	0	5
4	0	1 and 2	14	0	5
5	1	1 and 2	15	0	3
6	0	1 and 2	16	0	3
7	0	1	17	1	3
8	0	2	18	1 and 3	0
9	0	0	19	0	1
10	0	1	20	3	0

cination, Vaccine 1 makes the poorest showing. It would seem that this table requires little comment. It clearly demonstrates that the use of vaccines in this epidemic did not lessen the severity of the disease. We should add that pertussis in New York City as well as in our institution was characterized this year by its mildness. Indeed, we did not experience any fatality and but two cases of pneumonia. In an epidemic of this nature it would evidently be less valuable to judge the results of treatment by considering the number of mild cases, than by noting those of exceptional severity. This is all the more pertinent in a disease having the irregular symptomatology of pertussis, where mild cases can be differentiated with difficulty from the common cough of non-specific origin. In the tabulated cases it cannot be contended that treatment was not begun sufficiently early, as the first vaccination was in almost every instance administered during the first week of the disease. This is as early as we can hope to be able to employ vaccines in the course of any disease. Moreover, a glance at Table 2 (composed of cases which developed pertussis notwithstanding prophylactic inoculation) shows that where these early inoculations failed to ward off the disease, its severity was not mitigated; that in numerous instances not only did the vaccine fail to prevent infection, but that it did not shorten or mollify the course of the illness. In no instance, however, did any harmful local or systemic reaction follow the injection of vaccine. In this connection we may add that all attempts to employ the vaccines for diagnostic purposes proved futile, whether as a cutaneous, intracutaneous or subcutaneous test.

TABLE 5

COMPLEMENT FIXATION TESTS

Name	Date of Onset	Type of Vaccine	Date Vaccinated	Severity of Disease	Fixation Test, June	Bordet-Gengou Bacillus
F. G.	3/ 4/14	3	3/10/14	+++	+++	+
G. S.	3/22/14	3	3/10/14	+++	+++	+
E. S.	2/ 4/14	2	2/25/14	+++	±	+
D. F.	3/ 5/14	1 and 3	1/12/14 3/20/14	+++	±	+
A. V*.	5/15/14	3	2/ 5/14	+	±	–
G. H.	5/ 8/14	3	3/24/14	++	+++	–
P. B.	4/20/14	5	4/14/14	++	+	–
L. F*.	5/15/14	5	4/14/14	+	+	–
H. B.	4/22/14	3	4/17/14	+++	++	–
C. C.	3/ 9/14	3	3/ 9/14	+++	+++	–

*Pertussis doubtful.

The complement-fixation test of the serum was carried out on ten cases, using the Bordet-Gengou bacillus as antigen. These tests were made by Miss Olmstead of this laboratory, whom I wish to thank in this connection. The number of cases is far too few to allow of a statement of the value of this reaction in pertussis. In general, however, the results would seem to show that this reaction is present for some months after the cessation of all symptoms. Its chief interest is in connection with the still mooted question of the specificity of the Bordet-Gengou bacillus. It is for this reason that the cases are appended in a table (Table 5).

CONCLUSIONS

We have reported on the use of a series of vaccines in an epidemic of pertussis occurring in an institution for young children. An occasion of this nature furnishes exceptional opportunity to observe this disease, which is characterized by an indefinite onset and an irregular course, and is furthermore rarely adaptive to hospital study.

What deductions are to be drawn from the observations which we have recorded? It would seem clear that none of the four vaccines, including an autogenous strain, was of value in curing or tempering the disease in the epidemic which existed in our institution. That this failure was not due to tardy inoculations is emphasized by the circumstance that many of the severest cases received not only early active treatment but also prophylactic vaccination. No other conclusion seems possible from a review of the epidemic of 85 cases, and a comparison of the severity of the disease among the vaccinated and the unvaccinated. This opinion is based solely on our experience in this epidemic and does not preclude the possibility that a different dosage of vaccine, or treatment with a modified vaccine, may prove to possess curative properties.

The use of the vaccines in prophylaxis seemed by analogy to offer greater promise, in view of the established fact that other vaccines, for example the typhoid vaccine, possess prophylactic although no curative power. This seems to some extent, although to a far less degree, to be true of pertussis vaccine. In view of the fact that twenty children developed pertussis in spite of the prophylactic treatment, we cannot compare its protective value to that of typhoid vaccine. However, the proportion of unvaccinated children who developed pertussis so greatly exceeded the number of the vaccinated who developed the disease, that we conclude that the vaccine has protective value in a certain percentage of cases, and that it should be employed in institutions and in families to prevent the spread of this infection.

THE EFFECT OF QUININ ON RABIES IN RABBITS AND DOGS

Charles Krumwiede, Jr., and Alice G. Mann

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About a year ago, Moon[1] reported the recovery of three dogs treated with quinin after the development of symptoms of rabies. We attempted to repeat his work, but because of the results obtained the work was discontinued. The recently reported, negative results of Cumming,[2] who found that quinin had no prophylactic nor curative effect in rabbits, and the equally negative results of Frothingham and Halliday,[3] using rabbits, seem to warrant the addition of our results.

A series of rabbits were inoculated with fixed virus through a trephine opening, and the injection of quinin was started on different days thereafter. The quinin was the bisulphid, and a 1-8 solution in water was used. The inoculations were made August 9, 1914, and Rabbits 1 and 2, which received no quinin, became paralyzed August 15, and died August 17. Rabbits 3, 4 and 5, which received 2 c. c. of the quinin solution on August 14, as well as Rabbits 6 and 7, which received 2 c. c. of quinin solution on each day from August 11-15, also became paralyzed on August 15, and died August 17. The remaining rabbits in the series were killed by the quinin; 12 c. c. killing in a few hours, 8 c. c. over night, and 4 c. c. after several doses.

No difference could be noticed in any of the rabbits. The treatment was started so early that it would be expected to manifest some influence if it possessed any potency.

Six dogs were then inoculated with rabies and treatment with quinin was started on a companion dog as soon as any symptom developed in a dog of similar weight. The dogs were inoculated intracranially with street virus on August 9, 1914.

Dog 1, a pup, became depressed August 19; paralyzed August 20; died the next day.

Dog 2, a black and tan, weighing 7 pounds, showed no symptoms on August 21 and received 15 gr. of quinin sulphate, in capsules of 5 gr., every day from August 21 to September 2, when it appeared well and lively; September 12, it showed weakness of the hind legs; September 13, weakness and a high-pitched bark; September 15, death.

Dog 3, 24.5 pounds, showed weakness August 24; became paralyzed August 25; died the next day.

Dog 4, weighing 16.25 pounds, showed no symptoms on August 25 and was given 20 gr. of quinin sulphate in capsules of 5 gr.; August 26, it received 12 c. c. of a solution quinin bisulphate ⅛; August 28, there was spastic paralysis, and it received 10 gr. of quinin sulphate and 16 c.c. of the bisulphate solution; on August 29 it received 15 gr. of the sulphate and 16 c. c. of the solution; August 30, died.

Dog 5, terrier, 18.25 pounds, appeared excited on August 25 and received 20 gr. quinin sulphate; August 26, it received 5 gr. of quinin sulphate; August 27, 10 c. c.; August 28, 16 c. c.; died August 29.

Dog 6, mongrel, 18 pounds, acted suspiciously August 24; August 25, received 25 gr. of the sulphate, it was excited and rather weak; on August 26 it received 30 gr.; August 27, 40 gr.; August 28, 25 gr.; August 29, it seemed somewhat improved and received 30 gr.; again on August 30 and 31; on September 1 its condition was good, although there was a slight weakness, and it vomited about half of the quinin given that day, namely 30 gr.; found dead, September 2.

In all dogs, the brain contained typical Negri bodies, and material from each dog produced rabies in guinea-pigs, indicating that the virulence was not destroyed by the treatment.

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[1] Jour. Infect. Dis., 1913, 13, p. 165.
[2] *Ibid.*, 1914, 15, p. 205.
[3] Jour. Med. Research, 1914, 30, p. 275.

The only possible effect of quinin in these experiments is the remission in Dog 6 and the prolongation of the period of incubation in Dog 2.

SUMMARY

Of four dogs treated with quinin, one showed a prolonged period of incubation and another showed a remission in the course of the disease, in either case, however, not beyond variations in the natural course of the disease. The fact that these variations occurred in the two of four dogs receiving the largest amounts of quinin is probably only a coincidence. No influence was observed on the period of incubation in rabbits.

WHOOPING COUGH

Its Treatment and Prophylaxis, Based on the Bordet-Gengou Etiology

Paul Luttinger

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The work reported here is part of an extensive investigation of the etiology, diagnosis and treatment of pertussis undertaken by the Bureau of Laboratories of the health department in collaboration with some private practitioners. It is a tentative report on both the curative and prophylactic worth of the vaccine treatment.

In 1906, the two Belgian bacteriologists, Bordet and Gengou, communicated to the Academy of Medicine of Brussels, the discovery of a bacillus which they regarded as the cause of whooping cough. It was an influenza-like, polar staining organism, somewhat larger than Pfeiffer's bacillus, which could be isolated only on a special blood medium, from sputum coming from pertussis cases in the catarrhal or the first week of the paroxysmal stage of the disease.

During the spring and summer of 1913, Dr. Anna W. Williams (1), of the city research laboratory, found the Bordet-Gengou bacillus in the sputa of whooping cough patients with enough constancy to warrant a preliminary experiment by the department of health, with the vaccine treatment. Although Bordet himself had not reported any success with specific therapy, there were several American clinicians who had obtained encouraging results with small doses of commercial vaccine.

Preparation and Types of Vaccine

The following method has been most frequently used by Doctor Povitzky in making the vaccines: a Forty-eight hour culture on the special Bordet-Gengou medium is scraped off with normal salt solution. It is then shaken three to four hours in the shaking apparatus, after which the emulsion is heated for one hour at 58° to 60° C. and standardized according to Wright's method; 0.25 per cent. carbolic acid is added as a preservative and the vaccine is put up in 10 c. c. vials containing 100 million, 500 million and one billion killed bacteria to each c. c. Vaccine No. 7, instead of being heated for one hour was left in the incubator at 56° C. over night.

The antigens, referred to in the tables, were made by Doctor Povitsky in two days: First, by leaving the bacillary emulsion for eighteen hours in the incubator, centrifugating and using the supernatant fluid; secondly, by using the whole emulsion without centrifugation.

The serobacterins were sent for trial by a commercial firm and were found rather disappointing in the few cases in which they were used. Not much could be expected from a shotgun mixture of vaccines of which the specific organism formed only one-seventh of the dose, the remainder consisting of equally "sensitized" influenza bacilli, staphylococci, streptococci, pneumococci and the catarrhalis group.

The stock vaccines were made from strains isolated at the research laboratory, with the exception of Nos. 1 and 2, which contained a strain obtained from a commercial house. No. 1 contained three influenza strains.

The various strains entering into the composition of the vaccines were as follows:

Vaccine No.	Strains.
1	Com., 33, 35, 37
2	Com., 1, C.
3	55, 93, 95, 98
6	55, 93, 95, 98
7	55, 93, 95, 98
8	154, 155, 163

With the exception, perhaps, of vaccine No. 8, which seemed to be slightly less effective, all were about equally potent.

Method of Administration

The vaccines are given subcutaneously with an ordinary hypodermic syringe. The skin over the biceps is rubbed with a piece of cotton soaked in alcohol, and provided that the needle has been sterilized, there is no fear of infection. Large doses, to infants, may be given in the gluteal region.

Doses and Intervals

At the beginning, an initial dose of fifty million used to be given, which was doubled every three or four days. Now, we start with 250 million, which is doubled every other day, provided that there is no reaction; *i. e.,* the second dose is double the first, the third is twice as large as the second, and the fourth twice as large as the third, or two billion.

To prophylactic cases only three injections of 500 million, one billion and two billion respectively are given every three days.

Reaction

In nearly 3,000 injections given, I have not seen a single severe reaction. By severe reaction I mean one showing a temperature of 103° to 104° F. malaise, vomiting, diarrhea, and chills. A few moderate reactions with malaise, headache, and a temperature not over 100.5° F. have occurred, but in most of them it could be ascribed to gastrointestinal or other disturbances. It is well, however, to warn the parent against a possible local or slight general reaction.

There is absolutely no danger of anaphylaxis. If I make this apparently superfluous statement, it is because several physicians who received their medical training before the era of vaccines and serums, confound the two terms and often call up to ascertain, before using the pertussis vaccine, whether there are any records of anaphylactic shock following its administration.

Preliminary Series

The bulk of the 107 cases in our preliminary series were treated in the fall of 1913, at St. Joseph's Orphan Asylum, through the courtesy of Dr. William J. Bradley, the attending physician, to whom, as well as to the Sister Superior and Sister Magna, I am under deep obligation. Cases treated at the Lincoln Hospital, the Kingston Avenue Hospital, the New York Foundling Hospital, and in private practice by Dr. Albert Vander Veer, Jr., Dr. S. Ginsburg, Dr. Joseph O'Dwyer, Dr. De Castro, and myself are also included. Of these only forty-five have complete histories besides the twenty-five treated prophylactically.

The result of the treatment of the forty-five cases may be summarized as follows:

DURATION OF WHOOP

Less than one week	6.6
One to two weeks	8.8
Two to three weeks	13.3
Three to four weeks	22.2
Four to five weeks	18.1
One to two months	26.6
Over two months	4.4

Sixty per cent. had stopped whooping within one month. If we adopt Holt's figure of thirty days as the average duration of the paroxysmal stage in pertussis, we may consider these cases as only slightly, if at all, benefited by the vaccine treatment. I must mention the fact, however, that cases treated with the ordinary whooping cough mixtures had been whooping for forty-six to 180 days at the asylum. During an epidemiological investigation conducted in the spring of 1914, I found the average duration of the whoop to have been two months in the Borough of Manhattan. Only 26.7 per cent. had terminated within one month. Thus, by comparison we may ascribe some beneficial effect of the vaccine on the cases treated. The remaining cases, of which we have no complete records, were rather discouraging.

The pertussis vaccine did not seem to have any effect on whooping cough patients treated by the house staff of the New York Foundling Hospital at the suggestion of Dr. Matthias Nicoll, Jr. At the same time, Doctor Hess (2) was trying the laboratory stock vaccines and some autogenous vaccines at the Hebrew Orphan Asylum with mediocre therapeutic results, but with some prophylactic success. Doctor Hartshorn and Doctor Moeller (3), at the Roosevelt outpatient department, did not find our vaccines particularly effective. In private practice, however, they seemed to give some gratifying results to Doctor O'Dwyer, Doctor Ginsburg, and Doctor De Castro. Relatively small doses were given.

The twenty-five prophylactic cases were all treated at St. Joseph's. Twenty-three were given three injections of 100, 200, and 500 million respectively, at intervals of three or four days. No patient contracted the disease. One child who received one injection and persistently refused to submit to another, began to whoop about ten days later. The twenty-fifth or control case which received three injections of alcohol manifested a whoop a fortnight later.

This experiment loses a good deal of its significance, however, on account of the lack of information regarding the exact time of exposure. Many had not been exposed at all until after the treatment, while others presumably had mingled with whoopers from one day to four weeks. In view of the fact that during the epidemiological investigation referred to, we had found that 29.3 per cent. of children did not contract the disease although in intimate contact with the whooping members of the family, we must assume that probably a fraction of the St. Joseph's cases were naturally immune to the disease, and their subsequent freedom from whooping cough should not be ascribed to the vaccine.

Dr. S. Ginsburg, using the laboratory vaccine in doses of fifty million, reports two cases which failed to respond to vaccine prophylaxis. Dr. A. Conrad was kind enough to prepare a synopsis of pertussis cases treated with our vaccines at the Metropolitan Hospital. The average total dose was 355 million and the average total duration of the nineteen cases treated by him was twenty-eight days. This duration includes the stage before the paroxysms, and is shorter than that of our cases at the whooping cough clinic.

THE WHOOPING COUGH CLINIC

In order to obtain a more uniform view upon the subject as well as more abundant clinical material for bacteriological investigation, the department organized, in the summer of 1914, the whooping cough clinic at Sixteenth Street and Avenue C.

The facilities for the rational treatment and control of pertussis have not been, as all are well aware, any too numerous in New York. With the exception of one ward at Bellevue Hospital for complicated cases, and a few beds at the Metropolitan Hospital on Blackwell's Island, there are no hospital accommodations for severe cases of pertussis. The few dispensaries which consent to treat whooping cough do not fulfill all the requirements for the protection of other children, such as special clinics with separate entrances, etc. In the vast majority of the outpatient departments, the whooper is looked upon as a pariah and treated accordingly. Thus an anomalous situation, a kind of vicious circle, has been created for the sick poor. Applying to the dispensary for treatment, the mother is told to go home without delay and keep the child indoors until the whoop stops; at the same time she is given a card with printed instructions enjoining her to have the child out in the fresh air as much as possible. When the paroxysms become alarming, the lodge physician is sent for and the overworked contract practitioner is only too glad to diagnose whooping cough and advise the family not to call him again because, as they well know, there is no cure for the disease.

The whooping cough clinic, as evidenced by the increasing number of patients referred for treatment by private physicians, seemed, therefore, to have filled a long felt want. Recent criticisms in certain quarters regarding the ever-widening sphere of activities of the department of health, induce me to point out the fact that the cases treated at the clinic are either directly recommended by physicians or have no medical attendance at all. In a case of doubt, the physician who reported the case is invariably called up and his consent obtained before treatment is given.

Furthermore, in her follow up work, the clinic nurse visits every patient at one time or another, and reports on their economic status, thus preventing imposition.

PERTUSSIS CASES TREATED AT THE CLINIC

Of the 376 cases treated at the whooping cough clinic from August 10 to December 31, 1914, only a fraction is available for comparative study. The records show that they belong to the following groups:

	Cases Treated	Cases Tabulated
Still active	10	....
Negative	66	....
Doubtful	20	20
Drugs and vaccines	28	....
Incomplete	4	....
Prophylactic	34	34
Drugs	76	35
Vaccines	138	115
Total	376	204

NEGATIVE CASES; DIAGNOSIS

The negative cases, forming 17.5 per cent. of all those treated, came in with the diagnosis of pertussis, which, upon further examination, proved to be erroneous. Most of them were mild bronchial infections which improved rapidly under appropriate treatment. A few had tuberculosis, asthma, croup, and one adult had a subacute pharyngitis from excessive tobacco smoking. It requires a good deal of cross examination to get a history of any value from a dispensary patient. The mother will often exaggerate the symptoms in order to gain the physician's sympathy and, thereby, obtain " stronger " medicine; or she may try to minimize them if the youngster is anxious to go to school and is a general nuisance around the house. At other times a parent will answer " yes " or " no " according to a passing whim without serious regard to truth. Thus the records have to be often corrected to agree with facts subsequently discovered. When asking the mother whether the child was whooping, I found it best to illustrate the question by producing the specific sound. This is especially important when the parent speaks little or no English. The same may be said of vomiting, which is to be differentiated from the expelling of the plug of mucus and from mere regurgitation of food, which is common in healthy infants. The limitations of dispensary treatment made it almost impossible to ascertain the relative severity of the disease. In that respect private practice is just as unreliable. What to a young mother seems the severest attack imaginable, appears to the experienced matron as trifling. The father who is kept awake during the night by a few well distributed paroxysms of coughing is liable to describe his child's case as a " terrible one," while the severest attacks, if they occur during the day, when he is not at home, will leave him indifferent. A common standard of comparison could only be established in a hospital where the cases are under constant observation by the house staff.

The diagnosis of pertussis is made on the evidence of the whoop or paroxysmal cough with vomiting. The isolation of the Bordet-Gengou bacillus from the sputum clinches the diagnosis in doubtful cases, but a negative sputum does not exclude whooping cough. On account of other Gram negative bacilli very frequently found in the sputa of whoopers, one cannot diagnose pertussis from the bacterioscopic picture alone. The sputum must be planted on a special blood-agar-glycerin-potato medium and isolated from other organisms having the same morphological and staining characteristics.

The complement fixation test, notwithstanding statements to the contrary, has not proved, so far, to be of any practical value in the diagnosis of pertussis for therapeutic indications. In over a hundred cases studied at the research laboratory by Miss Olmstead and myself, we found positive complement fixation in about 50 per cent., and not earlier than the fourth day of the paroxysmal stage, *i. e.*, at a time when anybody could make the diagnosis if it depended on the whoop.

These negative cases are probably responsible for the extremes of opinion regarding vaccine treatment entertained by private physicians who treat a limited number of cases. A child with a nonspecific cough accompanied by regurgitation and magnified by a timorous parent into paroxysmal attacks with vomiting, is apt to improve " miraculously " after the second dose of vaccine, while a severe bronchitis simulating whooping cough will respond to vaccine treatment as much as to the same quantity of distilled water.

Doubtful cases. The same considerations may be applied to the twenty doubtful cases. From the appended Table I, showing both drug and vaccine treatment, it would not be wise to draw any definite conclusion as to the superiority of one over the other. Cases LIX and CCXXXII, for instance, had a total duration of the whoop of sixteen and twelve days respectively, which would have been ascribed to vaccine therapy if Case XLVII, treated with antipyrine, had not had an equally short paroxysmal stage.

It is possible that some of these doubtful cases were abortive forms of the disease. When testing a therapeutic remedy, however, one cannot be too careful in the diagnosis, and doubtful cases running an atypical course cannot form a basis to such an investigation.

CASES TREATED WITH DRUGS

Of the seventy-six cases treated with drugs, only thirty-five have been tabulated. The reason the others were omitted is the fact that the whoop had lasted longer than in the average case at the time they came for treatment. In such instances it is difficult to tell whether the paroxysms would have disappeared by themselves, or had been affected by the treatment. What conclusions, for instance, could one draw from a case of whooping cough which had lasted for eight weeks and in which the whoop stopped after three weeks' treatment? A basis of comparison with the vaccine cases had to be found and I have selected, somewhat arbitrarily, twenty-one days as the longest duration which would admit of comparative study. This limit allows nine days during which the drug or vaccine can be effective, and thus terminate the whoop within the average duration of one month.

In adding up the pertussis cases of less than three weeks' duration, treated with drugs, we find an average of *forty days*, or ten days more than the authorities allow for the paroxysmal stage of whooping cough.

Most of the cases were treated with an antipyrine mixture of the following composition:

℞	Antipyrini	0.065—gr. j;
	Sodii bromidi	0.200—gr. iij;
	Syrup aurantii florum, ad	4.00—ad ʒi.

M. ft. mistura. Sig.: This dose may be given to a child of one year as often as every two hours.

Children take the foregoing mixture readily on account of its sweet taste and agreeable flavor. The creosote mixture had to be discontinued, owing to its gastrointestinal disturbances which even small quantities were liable to produce.

CASES TREATED WITH DRUGS AND VACCINES

For the same reason as the doubtful ones, we cannot include these cases in our considerations regarding either the worth of antipyrine or vaccines. They are mostly therapeutic failures from either point of view. Many had complications and their attendance at the clinic was fitful. Some, started on antipyrine, were put on vaccine at the urgent request of the parent who had happened to see some vaccine cases improving more rapidly. Others were put on vaccines when a rise of temperature or other symptoms made us fear the onset of pneumonia. Still others, started on antigen No. 7, or too large doses of stock vaccines which produced some general reaction, had to be put on drug treatment to soothe matters.

TABLE I
Doubtful Cases of Pertussis

Serial No.	Case No.	Age in Years	Sex	Duration of Cough Before Whoop	Duration of Whoop Before Treatment	Vomiting	Treatment			Total Duration of Whoop or Cough	Remarks
							Type of Vaccine	No. of Injections	Total Dosage (in Millions)		
1	59	8 6/12	M.	Parox. cough 7 days	......	−	6	1	500	16 days	Exposed to pertussis.
2	158	15	F.	7 days	......	+	Anti-pneumococcus 7 & 8	3	3,500	35 days	Discharged with a slight cough. Sputum negative. Treated with creosote in addition to vaccine.
3	192	1	M.	90 days	5 days	−	6	1	250	105 days	Brother of 191, see below.
4	200	5	F.	2 days	......	−	8	3	1,750	70 days	Treated with antipyrine also. Discharged unimproved. Refuses further treatment.
5	201	3	F.	Parox. 2 days	......	−	8	3	1,750	?	Sister of 200. Also treated with antipyrine. Discharged unimproved.
6	232	3 6/12	M.	3 days	......	−	8	1	500	12 days	Sputum negative.
7	325	1 9/12	M.	Parox. 10 days	?	−	8	4	3,750	34 days	Sputum negative. Discharged improved.
8	47	4/12	M.	Parox. 7 days	? 7 days	−	Antipyrine mixture			14 days	
9	154	3 2/12	M.	21 days	? 7 days	−	Creosote mixture			35 days	
10	189	8	M.	7 days	......	−	Antipyrine mixture			70 days	Had pertussis three years ago.
11	190	5	F.	3 days	......	−	Antipyrine, also creosote mixture			63 days	Had pertussis three years ago.
12	191	2 4/12	M.	90 days	? 5 days	−	Antipyrine mixture			105 days	
13	195	5 7/12	M.	14 days	......	−	Antipyrine mixture			?	Sputum negative. Discharged, only little improved.
14	196	3 5/12	M.	21 days	......	−	Antipyrine mixture			35 days	Sputum negative. Brother of 195. Discharged with occasional cough.
15	197	6/12	F.	10 days	......	−	Creosote mixture			30 days	Bronchitis. Discharged with slight occasional cough. Sister of 195 and 196.
16	219	1 6/12	F.	14 days	14 days	+	Creosote mixture			35 days	
17	249	2 8/12	F.	7 days	90 days	−	Antipyrine mixture			105 days	Had pertussis before. All children (5) in family have pertussis.
18	291	2 3/12	F.	12 days	......	+	Antipyrine mixture			18 days	
19	301	3/12	M.	7 days	? 35 days	−	Antipyrine mixture			70 days	Discharged, coughing when excited only.
20	318	6	F.	?	......	+	Antipyrine mixture			?	Answers unreliable. Stopped coughing after 22 days of treatment.

TABLE II

PERTUSSIS CASES TREATED WITH DRUGS

Serial No.	Case No.	Age in Years	Sex	Duration of Cough Before Whoop	Duration of Whoop Before Treatment	Vomiting	Treatment	Total Duration of Whoop or Paroxysmal Cough	Remarks
1	1	1 5/12	M.	7 days	21 days	+	Antipyrine mixture; later creosote mixture	45 days	Creosote mixture seemed to aggravate.
2	2	3	M.	7 days	7 days	+	Antipyrine mixture; later creosote mixture	45 days	Creosote mixture seemed to aggravate.
3	4	4 6/12	F.	7 days	4 days	±	Antipyrine mixture	31 days	
4	7	3 6/12	F.	28 days	17 days	+	Antipyrine mixture	40 days	
5	21	3	F.	Parox. 14 days	......	−	Antipyrine mixture	42 days	Alternately improving and relapsing.
6	24	1 5/12	F.	7 days	14 days	−	Antipyrine mixture	35 days	
7	71	3	F.	21 days	21 days	+	Antipyrine mixture	28 days	Improving at time of first treatment.
8	91	4	F.	14 days	21 days	+	Antipyrine mixture	42 days	Conjunctivitis.
9	95	2	M.	14 days	21 days	+	Antipyrine mixture	28 days	
10	98	1 7/12	M.	28 days	14 days	+	Antipyrine mixture	28 days	
11	111	4 3/12	M.	28 days	14 days	+	Antipyrine mixture	18 days	
12	130	4	F.	7 days	21 days	±	Antipyrine mixture	38 days	
13	131	3	F.	7 days	21 days	+	Antipyrine mixture	38 days	Sister of 130. Improving at time of first treatment.
14	138	4	M.	7 days	14 days	+	Antipyrine mixture	21 days	
15	145	4	F.	21 days	14 days	+	Creosote	38 days	One child in family died of pertussis pneumonia.
16	163	6	F.	7 days	14 days	+	Antipyrine, also creosote	35 days	
17	169	3 6/12	M.	7 days	14 days	+	Antipyrine mixture	35 days	Whoop comes back every time medicine is stopped.
18	181	5	F.	7 days	21 days	+	Antipyrine mixture	38 days	
19	185	5 6/12	F.	7 days	14 days	+	Antipyrine mixture	56 days	Father died of tuberc. three years ago.
20	198	2	F.	7 days	7 days	+	Antipyrine and creosote mixture	21 days	
21	206	8/12	F.	......	21 days	+	Antipyrine	42 days +	Discharged in care of private physician, still whooping and vomiting.
22	211	4 5/12	M.	7 days	21 days	+	Creosote and antipyrine	45 days	
23	212	3	M.	7 days	21 days	−	Antipyrine	25 days	Getting well (stopped vomiting) at time of first treatment.
24	213	6	F.	7 days	17 days	−	Antipyrine	25 days	Getting well (stopped vomiting) at time of first treatment.
25	214	9	M.	7 days	17 days	−	Antipyrine	25 days	
26	220	4 5/12	M.	21 days	10 days	−	Antipyrine mixture	31 days	Transferred to private physician, still whooping.
27	268	3	F.	Parox. 17 days	......	+	Antipyrine mixture	56 days	Improving at time of admission.
28	284	5	M.	7 days	21 days	+	Antipyrine mixture	60 days	
29	292	6	F.	7 days	16 days	+	Antipyrine mixture	41 days	Brother of 293 and 295. See vaccine list.
30	307	2 6/12	M.	14 days	21 days	+	Antipyrine mixture	31 days	
31	308	4	F.	Parox. 10 days	......	−	Antipyrine mixture	31 days +	Discharged unimproved.
32	343	10/12	F.	Parox. 7 days	......	+	Antipyrine mixture	14 days	
33	251	8/12	F.	Parox. 14 days	......	+	Antipyrine mixture	70 days	Began to whoop 5 weeks after first treatment. Actually whooped for 3 weeks.
34	252	3 6/12	F.	14 days	21 days	−	Antipyrine mixture	42 days	Sister of 251 and 253. Improving at time of admission. Stopped vomiting.
35	253	5	M.	21 days	......	−	Antipyrine mixture	42 days	Began to whoop 2½ weeks after admission.

TABLE III.

Pertussis Cases Treated with Vaccines

Serial No.	Case No.	Age in Years	Sex	Duration of Cough Before Whoop	Duration of Whoop Before Treatment	Vomiting	Treatment			Total Duration of Whoop	Remarks
							No. of Injections	Type of Vaccine	Total Doses (in Millions)		
1	9	1 3/12	M.	4 days	......	–	3	7	1,600	11 days	Sputum positive. Began to whoop two days after admission.
2	10	3	F.	?	8 days	±	2	7	300	16 days	
3	13	1 5/12	M.	?	11 days	–	1	7	100	14 days	Brother of 10.
4	14	3 6/12	F.	14 days	7 days	+	3	7	1,600	27 days	
5	15	1 8/12	M.	7 days	7 days	–	3	7	1,550	23 days	Moderate reaction. Vomited later.
6	20	2	M.	14 days	1 day	–	5	7	3,800	14 days	Vomited two days after admission. Bronchitis.
7	22	1 2/12	F.	270 days	14 days	±	2	7 also Pn.V.	550 75	19 days	Chronic bronchitis.
8	23	4 6/12	F.	Parox.	1 day	–	4	7 also Pn.V.	3,600	22 days	Sputum positive. Vomited seven days after admission
9	27	3 10/12	M.	?	3-4 days	–	1	6	100	16 days	
10	28	1 3/12	F.	?	7 days	+	1	6	100	19 days	
11	33	1 5/12	F.	21 days	3 days	–	2	Antigen	500	14 days	Had stopped whooping. Began again eleven days later.
12	34	1 8/12	F.	7 days	2 days	+	1	6	50	11 days	Bronchitis; later developed pneumonia from which she recovered. Moderate reaction.
13	37	2	M.	7 days	Parox. 7 days	+	1	7	1,000	18 days	Slight reaction.
14	38	12	F.	14 days	14 days	+	2	7	3,000	24 days	Sister of 37.
15	41	3	M.	8 days	6 days	–	4	Pn.V. also 7	200 6,100	13 days	One of the children contracted pertussis three weeks after.
16	43	11/12	M.	Parox. 14 days	1 day	+	2	7	1,500	13 days	
17	46	1 2/12	M.	Parox. 7 days	......	–	4	7	3,750	14 days	
18	49	7	F.	7 days	14 days	+	1	7	500	17 days	
19	51	4	M.	Parox. 7 days	7 days	+	1	7	500	15 days	
20	53	2	M.	......	Parox. 7 days	–	3	7	3,500	28 days	
21	57	2 6/12	F.	3 days	......	+	4	7 also Pn.V.	5,875 75	45 days	Moderate reaction. In the same house there are children whooping four to six months.
22	58	4 2/12	F.	7 days	4 days	+	3	7	3,500	26 days	Sister of 57.
23	60	1/12	F.	7 days	3 days	–	1	7	350	7 days	
24	61	4	F.	14 days	7 days	+	2	6	1,500	11 days	
25	64	7/12	M.	35 days	7 days	–	1	7	400	14 days	Moderate reaction.
26	67	1 1/12	M.	7 days	14 days	–	3	6	3,500	39 days	
27	68	4	F.	7 days	3 days	–	3	6	3,500	28 days	Sister of 67.
28	70	1 4/12	M.	......	Parox. 21 days	±	3	6	3,500	28 days	
29	72	4/12	M	7 days	21 days	+	3	6	3,500	33 days	
30	74	9/12	F.	7 days	14 days	+	4	6	7,500	31 days	

31	76	3 6/12	M.	7 days	14 days	+	3	6	3,500	22 days	
32	82	4 6/12	F.	......	14 days	+	2	6	1,500	25 days	
33	83	7	F.	......	21 days	+	1	6	500	26 days	
34	84	8	F.	......	21 days	+	1	6	500	26 days	
35	85	4	F.	......	21 days	+	1	6	500	26 days	
36	86	3	F.	......	21 days	+	1	6	500	26 days	
37	88	9/12	F.	7 days	7 days	+	1	6	500	14 days	
38	89	4 2/12	M.	7 days	10 days	+	1	6	400	25 days	
39	92	2	F.	7 days	1 day	—	4	2Serobacterin 2 6	C&D 3,000	24 days +	One sister had pertussis-pneumonia; serobacterins aggravated condition. Contracted P. P. in a hospital and died.
40	94	5/12	M.	14 days	21 days	+	1	6	500	26 days	Moderate reaction.
41	97	1 8/12	M.	7 days	7 days	+	2	Pn.V. also 6	1,500 +75	12 days	Had bronchitis during all winter.
42	105	2 6/12	F.	4 days	10 days	+	4	2Serobacterin 2 6	A+B 1,500	44 days	After having stopped for two weeks whoop returned.
43	106	11/12	M.	......	Parox 21 days	—	3	7	3,500	42 days	Vomited during treatment.
44	107	2	F.	......	21 days	+	2	7	1,500	28 days	Epistaxis frequent.
45	108	1/12	F.	7 days	7 days	+	2	6	1,500	19 days	
46	109	2 6/12	F.	14 days	14 days	+	3	6	3,500	55 days	
47	110	4/12	M.	2 days	3 days	—	3	6 & 8	5,500	44 days	Brother of 109.
48	113	3	M.	7 days	14 days	+	4	Serobacterin	½A BC&D	36 days	Colored child.
49	114	4	F.	7 days	7 days	+	4	Serobacterin	½A BC&D	29 days	Colored child.
50	120	11/12	M.	......	Parox. 7 days	—	3	7	3,500	26 days	
51	122	4	F.	7 days	14 days	+	2	6	1,500	43 days	Child faints during attacks.
52	125	4/12	F.	7 days	7 days	+	3	1	3,500	31 days	
53	127	6	M.	7 days	21 days	+	1	6	500	30 days	
54	129	2	M.	7 days	14 days	+	2	6	1,500	36 days	
55	133	1 3/12	F.	7 days	17 days	—	3	7	3,500	41 days	
56	134	2 5/12	F.	23 days	14 days	+	3	1	3,500	25 days	
57	135	8	F.	28 days	1 day	±	3	6	3,500	15 days	
58	136	11/12	F.	7 days	7 days	+	2	6	1,500	16 days	
59	140	8/12	F.	84 days	21 days	+	6	Antigen 6 & 7	150	111 days +	Mother has syphilis in the tertiary stage.
60	150	9/12	F.	21 days	14 days	+	2	6	1,500	21 days	Almost asphyxiated during one attack. Artificial respiration employed.
61	153	3	F.	14 days	7 days	—	4	Antigen 7	3,750	18 days +	Vomited during treatment. Discharged unimproved.
62	152	2 6/12	M.	28 days	14 days	+	4	7	850	25 days +	Discharged unimproved. Mother prefers "gas house" treatment.
63	156	7	F.	7 days	21 days	+	3	3	1,750	30 days	Vaccine nine months old.
64	157	3	M.	14 days	7 days	+	3	3	1,750	16 days	Brother of 156
65	161	6/12	F.	......	7 days	—	3	8 also Pn.& P.V.	3,000 500	32 days	
66	162	3	F.	7 days	7 days	—	3	8 also Pn.& P.V.	3,000 500	36 days	Sister of 161.
67	165	9	M.	7 days	14 days	+	1	6	500	21 days	
68	166	4	M.	7 days	7 days	—	1	6	500	14 days	Cousin of 165 living in same house.
69	171	1 2/12	M.	3 days	21 days	+	2	6	1,500	28 days	An older brother whooped for 12 weeks.
70	174	3	F.	14 days	14 days	+	3	6 & 8	3,500	24 days	
71	175	6	F.	14 days	7 days	+	3	6 & 8	3,500	17 days	Sister of 174.

TABLE III. (*Continued*)

Serial No.	Case No.	Age in Years	Sex	Duration of Cough Before Whoop	Duration of Whoop Before Treatment	Vomiting	Treatment: No. of Injections	Treatment: Type of Vaccine	Treatment: Total Doses (in Millions)	Total Duration of Whoop	Remarks
72	180	2/12	F.	7 days	7 days	+	2	1 & 8	1,500	25 days	
73	182	1 11/12	F.	7 days	7 days	+	5	6-1-8	3,750	23 days	
74	183	2/12	F.	......	Parox. 7 days	+	5	6-1-8	10,750	19 days	Began to whoop 4 days after admission. Actually whooped only 12 days.
75	184	1 9/12	F.	7 days	4 days	+	4	6 & 8	7,500	25 days	Whooped hardly 8 days after first injection.
76	209	3	M.	7 days	7 days	+	3	8	1,750	14 days	
77	217	6/12	F.	14 days	14 days Parox.	+	3	8 also 7 +Pn.V.	750 1,000	26 days	
78	222	1 2/12	M.	7 days	1 day	+	2	8	1,500	5 days	
79	224	3/12	F.	2 days	4 days	+?	3	6 & 8	1,750	47 days	
80	229	1 8/12	M.	......	Parox. 14 days	–	2	8	750	23 days	
81	231	11/12	F.	......	Parox. 21 days	+	1	8	250	30 days	
82	233	5/12	F.	4 days	2 days	+	2	8	750	8 days	
83	234	1/12	F.	......	Parox. 7 days	±	2	8	300	11 days	One brother came for treatment in sixth week of whoop —47 days.
84	236	4 6/12	M.	14 days	7 days	+	3	8	1,750	17 days	
85	241	3	M.	......	Parox. 7 days	±	2	8	750	22 days	
86	243	6 6/12	F.	7 days	7 days	+	3	8	1,750	25 days	
87	245	6 6/12	F.	21 days	1 day	±	3	8	1,750	10 days	
88	246	3 2/12	F.	7 days	14 days	–	5	8 Pn. +P.V. 7	1,500 650	71 days	Developed double pneumonia after second injection. Complement fixation test positive.
89	254	2/12	F.	......	Parox. 14 days	±	1	8	500	42 days	
90	270	1 3/12	M.	7 days	14 days	+	3	8	3,500	37 days	
91	271	5	F.	7 days	14 days	+	3	8	3,500	22 days	Sister of 270.
92	273	3 6/12	F.	7 days	14 days	+	3	8	1,750	29 days	
93	274	1 7/12	M.	7 days	5 days	–	3	8	1,750	18 days	Brother of 273. } Mother expected disease to last 18 weeks.
94	281	3/12	M.	......	Parox. 8 days	–	3	8	3,500	24 days	
95	283	1	M.	7 days	8 days	+	4	8	3,750	38 days	
96	286	10/12	F.	7 days	7 days	+	3	8	1,750	12 days	This child had the severest attacks we have witnessed, also most frequent.
97	287	2 6/12	F.	7 days	14 days	+	3	8	1,750	19 days	
98	288	4	F.	7 days	21 days	+	3	8	1,750	26 days	Three children in family untreated. Whooped for about 90 days.
99	293	1	F.	......	Parox. 7 days	–	2	8	750	30 days	
101	295	5	M.	7 days	7 days	+	3	8	1,750	56 days	Chronic bronchitis. Brother of 293.
100	294	3 6/12	M.	7 days	7 days	+	2	8	750	23 days—	Admitted to our ward 3. Discharged improved, but still whooping.

102	297	3	M.	7 days	14 days	+	1	8	250	20 days	Brother of 296. Also admitted to ward 3. Discharged cured.
103	299	3 6/12	F.	7 days	7 days	+	3	8	1,750	24 days	
104	300	4 8/12	F.	7 days	7 days	+	3	8	1,750	24 days	Sister of 299.
105	306	6/12	F.	1 day	10 days	–	4	8	3,750	16 days	
106	315	5 6/12	F.	7 days	7 days	+	3	8	1,750	17 days	Sputum positive. Contracted disease 8 weeks after sister, 241, began to whoop.
107	323	3	M.	21 days	7 days	–	2	8	750	13 days	
108	326	9/12	F.	6 days	1 day	–	2	8	750	18 days	
109	320	1/12	F.	7 days	1 day	+	2	8	150	4 days	Sister of 323.
110	335	1/12	F.	......	14 days Parox.	+	1	8	200	21 days	
111	347	1 10/12	M.	7 days	7 days	+	1	8	500	24 days	
112	353	2 3/12	F.	2 days	4 days	+	3	8	1,750	9 days	Sputum positive.
113	354	11/12	F.	7 days	3 days	+	1	8	250	24 days	Eleven days after injection contracted pneumonia from which she recovered.
114	359	4	F.	2 days	14 days	+	4	8	3,750	30 days	
115	361	3 6/12	F.	7 days	7 days	+	3	8	1,750	14 days	

TABLE IV

PERTUSSIS PROPHYLACTIC CASES

Serial No.	Case No.	Age (in Years)	Sex	Duration of Exposure	Symptoms	Treatment			Result	Remarks
						No. of Injections	Type of Vaccine	Total Doses		
1	18	5/12	F.	8 days	Coughing for 3 days	3	7	1,600	Stopped coughing within 2 weeks. No whoop. No vomiting.	Brother of this patient seen in consultation with Dr. T. suffering from pertussis-pneumonia.
2	30	8	F.	14 days	Coughing for 7 days	3	7	5,100	No whoop. No vomiting. Stopped coughing after injection.	One sister has a very severe attack of pertussis. One brother, age 4 6/12, free from disease.
3	69	7	M.	14 days	. . . .	2	6	1,500	No symptoms.	One brother whoops for 2 weeks. Another 3 days.
4	77	2 6/12	M.	7 days	Coughing 7 days	1	1	500	No whoop. No vomiting.	One brother died of pertussis in a hospital. Moderate reaction. Mother refuses further treatment.
5	79	1 6/12	F.	14 days	Slight cough	3	6	3,000	No whoop. No vomiting. Cough stopped in a month.	
6	100	9	M.	42 days	"Croupy" coughing one week	1	6	500	No whoop. No vomiting. Stopped coughing 2 weeks later.	Moderate reaction. One brother died of tuberc.
7	102	1/12	M.	14 days	. . . .	3	6	3,500	No symptoms.	Slight reaction.
8	128	10/12	F.	21 days	Coughing 7 days	1	6	500	No whoop. No vomiting. Stopped coughing after injection.	Slight reaction. Sister and brother have pertussis for 2 and 3 weeks, respectively.
9	132	1/12	M.	?	Coughing 1 day	1	6	500	Began to whoop and vomit 12 days after injection.	Marasmus; died in a hospital 6 weeks later from intestinal complications and pneumonia.
12	146	6 4/12	F.	9 days	. . . .	1	6	500	No symptoms.	Sister (139) whooping for 9 days.
11	145	3 10/12	F.	9 days	. . . .	1	6	500	No symptoms.	Sister of 146.
10	144	2 6/12	M.	35 days	Paroxysmal coughing for 2 days	1	6	500	Cough stopped 3 days after injection.	Exposed to older brother.
13	148	1 2/12	M.	7 days	Coughing 5 days	1	6	500	Cough stopped 3 days after injection.	
14	149	4 6/12	M.	6 days	Coughing 3 days	2	6	1,500	Stopped coughing 4 days after last injection.	Vomited several times on day of second injection.
15	151	1 2/12	M.	14 days	Coughing 7 days	1	7	500	Cough stopped a few days later.	Slight reaction.
16	167	2	F.	7 days	Paroxysmal coughing 7 days	1	Pn. +7	500	Began to whoop 2 weeks after injection.	Older brother whooping for one week.
17	179	2/12	F.	5 days	Coughs and vomits 2 days	3	Pn. +7 also 8	3,500	No whoop. Stopped vomiting 5 days after last injection.	
18	199	4	F.	7 days	Coughing 5 days	3	8	1,750	Stopped coughing after last injection.	
19	202	3/12	M.	28 days	Paroxysmal coughing for 7 days	1	8	500	Stopped coughing 3 weeks after injection.	
20	208	1 2/12	F.	7 days	. . . .	3	8	3,500	No symptoms.	
21	218	4 6/12	F.	14 days	Coughing 10 days	1	8	500	No whoop. No vomiting.	
22	225	4	M.	4 days	Whooping? 4 days	2	6 & 8	1,500	Began to whoop and vomit.	

23	226	3/12	M.	4 days	Paroxysmal cough 4 days	2	6 & 8	1,500	Began to whoop and vomit.	Brother of 225.
24	237	2 6/12	F.	7 days	....	2	8	1,500	No symptoms.	Sister of 225 and 226.
25	244	1 6/12	F.	7 days	Coughing 4 days	1 (3)	8 8	500 (3,500)	Began to whoop 2 weeks after first injection.	Interval between first and second injection—16 days.
26	247	11/12	F.	14 days	Coughing 7 days	3	8	3,500	Stopped coughing 5 days after last injection.	
27	248	11	F.	14 days	....	2	8	1,500	No symptoms.	Sister of 247 and 298.
28	250	8/12	F.	2 days	Coughing 2 days	1	8	500	Stopped coughing fifth day after injection.	
29	272	3	F.	14 days	Coughing 4-5 days	3	8	3,500	Stopped coughing. No other symptoms.	Vomited between injections.
30	280	1 8/12	M.	14 days	Coughing 5-6 days	3	8	3,500	Stopped coughing after second injection.	Brother of 272.
31	298	8	F.	1 day	....	3	8	3,500	No symptoms.	Sister of 248 (see above). Difference in time of exposure due to absence from home.
32	309	2/12	F.	10 days	Coughing 2 days	Antipyrine	mixture		Began to whoop and vomit after 4 days' treatment.	Mother refused vaccine treatment.
33	319	2	F.	4 days	Coughing 2 days	1	8	500	Stopped coughing 3 days after injection.	Slight reaction. Mother calls the child's cough, whooping, because other child began in a similar manner and is whooping severely now.
34	330	6	M.	1 day	....	2	8	3,000	No symptoms.	

Only four of these cases had a paroxysmal stage of less than average duration. Case VIII started on creosote and finishing on vaccine, whooped for twenty-eight days. Cases LXXXVII and CXXVI, starting with vaccine, ended with creosote, having a paroxysmal stage of twenty-four and twenty-five days. Case CCLVI, after one injection of vaccine, refused further injections and was put on antipyrine, whooped for twenty-nine days; while Case CCLXXXII, requested antipyrine after submitting to three injections, and whooped for only eighteen days.

Pertussis Cases Treated With Vaccines (Table III.)

Of the 138 cases treated with vaccines, 115 came to us within the first three weeks of the paroxysmal stage. The average duration was twenty-five days. This figure could have been much reduced if such cases as CXL, for instance, whose mother has syphilis and whose low resistance can be guessed from the long duration of the cough (eighty-four days) which preceded the whoop. This child came to the clinic just on the twenty-first day of the whoop as if to test our impartiality. It whooped for more than 111 days and was finally discharged with an occasional whoop.

Nevertheless, the vaccine treatment seems to have decreased the paroxysmal stage, compared to the drug treatment, by over two weeks. What is more significant, however, than the shortening of the duration, is the prompt amelioration which follows the administration of the vaccine in nearly all cases. The whoop becomes milder and in the last weeks consists often of an occasional paroxysm only. In some cases, however, especially when a moderate reaction follows the first injection, a slight aggravation takes place in from twenty-four to forty-eight hours. The mother should be warned of such a possible aggravation if one cares to retain the patient.

The course of a pertussis case treated with vaccine is usually as follows:

1. Decrease in the severity of the night paroxysms.
2. Vomiting stops—severity of day paroxysms abates.
3. Night paroxysms stop—day paroxysms reduced in number.
4. At the end of first week of treatment, whoops are about twenty-five per cent. of their former frequency. Mother refuses further vaccine treatment, as she thinks that child will get well anyway.

The cases treated with antipyrine, as a rule, show first a decrease in the *number* not the *severity* of the paroxysms. Vomiting is apt to continue until or after the whoop stops in a rather abrupt fashion. Both are apt to return as soon as the medicine is withdrawn or on the least provocation due to a new "cold," gastrointestinal trouble, etc. Such relapses are rare in cases treated with vaccines.

The number of injections given exceeded three only in a few cases. As a rule the parent will refuse further "needle" treatment as soon as there is a marked amelioration in the character and number of paroxysms. This actually prevents us from ascertaining the full worth of the vaccine, and it is a drawback which could disappear only in hospital treatment when the patient is not subject to the parents' control.

Complications. Among our vaccine patients, four developed pneumonia. Three of these (aged eleven months, twenty months, and three years respectively) made perfect recoveries. The fourth (Case XCII, two years old) treated with a commercial, so-called sensitized vaccine, drifted away from the clinic, partly on account of the lack of improvement and partly because the mother who was at term could not bring the child for treatment.

We finally heard from the neighbors that the child had been taken to a hospital, where it contracted pneumonia and died.

In view of the fact that fully one-third of our vaccine patients were less than one year old, it is remarkable that we had no more pneumonia cases and that, with the exception of Case CXXXII, listed in Table IV, we had no other fatalities.

PROPHYLACTIC CASES (TABLE IV)

Of the thirty-four cases tabulated only nine could be called strictly prophylactic, *i. e.,* exposed to pertussis, but showing as yet no symptoms of the disease. The others had either a plain cough or a mildly paroxysmal one with vomiting, and the treatment was given with the view of aborting the disease rather than preventing an infection which already seemed to exist.

The nine strictly prophylactic cases gave histories of exposure from one to fourteen days. After receiving the three injections, they have remained without any symptoms up to date (two to four months after immunization).

The remaining twenty-five cases resulted as follows: Nineteen patients stopped coughing, during, or soon after treatment. One of these has no significance as he had been exposed forty-two days and probably would not have contracted the disease in any case. The vaccine was given to allay the mother's apprehension.

One case treated with antipyrine soon developed the whoop. In the remaining five (15.1 per cent.) the vaccine did not seem to be able to abort the disease. Of these, Case CXXXII (one month old and suffering from marasmus) referred to above, contracted pneumonia and was fatal.

That marasmus in itself is not incompatible with recovery from whooping cough, was illustrated by the case of Doctor Van Der Veer at the Lincoln Hospital. The three months old colored child weighing only seven pounds fourteen ounces made a perfect recovery under vaccine treatment and appropriate feeding, and was gaining weight at the end of a month.

SUMMARY AND CONCLUSIONS

If we admit the Bordet-Gengou etiology of pertussis, we must free ourselves from a few superstitions regarding whooping cough. One of these is the infectiousness of the paroxysmal stage, which, like that of the scales in scarlet fever, seems to have been unduly exaggerated. The fact that the Bordet-Gengou bacillus has been most often found in the sputum of the catarrhal and rarely later than the first week of the paroxysmal stage, points to the early part of the disease as the most infectious and there would seem to be no necessity for the child to be kept in the house for more than a week after the whoop appears. If accompanied by a competent guardian who takes care to collect the expectoration in a paper bag to be subsequently burned, and sees to it that the child does not whoop or sneeze in other children's faces, the pertussis patient may be allowed to be out of doors from the beginning.

The placarding of homes where pertussis exists and the wearing of arm bands by the whoopers which had been advocated by some pediatrists, also seems to be of doubtful utility in all except the acute cases. Not because such measures are annoying or humiliating to the individual sufferer, but on account of the impossibility of reaching the abortive cases, those in the catarrhal stage and the healthy carriers who are, perhaps, the most dangerous disseminators of infection.

The necessity of more whooping cough clinics and of a pertussis hospital has become more and more evident as these studies have progressed.

In view of the fact that the vaccines have given some encouraging results in the prophylaxis and treatment of pertussis, it would be advisable that private practitioners collaborate with the Bureau of Laboratories in a more extensive test of these vaccines. The necessary quantity accompanied by directions for administration and a history card will be given to any city practitioner on demand, who promises to report the results obtained.

In doubtful cases the sputum may be sent to the research laboratory for diagnosis. To be of any value, the sputum must be obtained early in the disease from a bronchus, not mere saliva from the mouth mixed with food particles. The characteristic sputum is obtained by inducing a paroxysm by tickling the child's fauces with a wooden tongue depressor and appears as a grayish white tenacious plug of mucus.

Many physicians seem to underestimate the seriousness of the disease and fail to report cases of whooping cough to the department. In a recent investigation we found that only about twenty-six per cent. of the cases in a limited area were reported. This figure is too high, as probably not more than ten per cent. are reported in greater New York. Vital statistics is the basis upon which rest all future plans of public sanitation, and I trust that the practitioners in the city will report promptly all cases of whooping cough occurring in their practice. The following conclusions may be drawn from this work:

1. Pertussis stock vaccines as prepared by the Bureau of Laboratories seem to have a prophylactic value when given in high doses.
2. In the treatment of pertussis, these vaccines seem to have shortened the duration and severity of the paroxysmal stage; the average duration of the whoop being twenty-five days, compared to forty days of those treated with drugs.
3. Further experiments with the view of obtaining more effective vaccines and a closer co-operation of the profession in public health education, may help in the eradication of pertussis, this scourge of childhood which kills yearly ten thousand American children.

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TREATMENT OF CASES OF EPIDEMIC MENINGITIS ILLUSTRATING THE IMPORTANCE OF FREQUENT REPETITION OF THE INJECTION

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The two most common mistakes in serum treatment of epidemic cerebrospinal meningitis seem to be giving too few doses of serum if the patient improves considerably after the first one or two injections, and failing to persist with the serum if the improvement is very slow. It has been the experience of the Meningitis Department during the past five years that it is rarely safe to give less than four doses of serum on consecutive days even if the improvement clinically is very rapid and the organisms disappear from the fluid after one or two injections. In cases that have been running on for some time and are evidently improving when first seen, one or two injections of serum are sometimes sufficient. Occasionally a case seen very early and clearing up quickly may be given only three injections. A case of average severity usually required from four to seven injections. It is safer to give the injections on consecutive days until it seems evident that the case is out of danger, than to skip a day or two when a slight improvement occurs, thereby giving the organism a chance to increase. Puncture for the relief of pressure may have to be done several times during convalescence. At such punctures, a little serum may be injected, especially if a large amount of fluid is withdrawn. In a small percentage of cases—from 5 to 10 per cent.—a large number of injections may be necessary before the termination of the case. A certain number of such cases terminate fatally.

The following cases recently treated by the Department illustrate very well the necessity of patience and persistence in administering serum when the progress is discouragingly slow.

CASE I.—H. M., young man of 19, seen first July 16, 1915; past history, negative; present illness, July 5th complained of headache and pain in back which resembled a mild influenza and cleared up in a few days, the patient being quite well until July 14th when the headache returned and vomiting occurred. There was also a chill. When examined on the 16th, he was delirious; there was marked stiffness of the neck; Macewen's and Kernig's signs were positive; the knee jerks were slightly increased; the pupils were equal and reacted to light; the pulse was 60 and regular; temp. 101.4; respiration Biot in type; no paralysis; no eruption. 50 c.c. of cloudy fluid was withdrawn by lumbar puncture and 20 c.c. of antimeningitis serum was injected. The fluid showed a great increase in cells, 95 per cent. being polynuclears; intracellular gram negative cocci by smear, albumen and globulin both greatly increased; no reduction of Fehling's. July 17th, condition somewhat better; temp. normal; still delirious. 45 c.c. very cloudy fluid—20 c.c. serum. Punctures were repeated and serum was administered on the 18th, 19th, 20th, 21st and 22d. Beginning the 19th, the amount of serum given was increased from 20 to 35 c.c., the amount of fluid drawn being at all times greater than this, and, until the 21st, exceedingly purulent; an inch or more of pus settling to the bottom of a large test tube containing 25 to 30 c.c. of fluid. That of the 22d was much clearer. There continued to be a pure culture of meningococci until the fluid of the 21st, though that of the 19th was scanty. Fehling's was not reduced until the

21st; that of the 22d showed good reduction. The albumen and globulin were always greatly increased. The temp. was normal beginning with the 17th. Much of the time there was great difficulty in swallowing owing to the extreme retraction of the head, and it was necessary to give small quantities of food at frequent intervals. On the 23d and the 24th, as the fluids had been sterile for two days and as the patient seemed considerably better, no punctures were done. On the 25th, the puncture was done to relieve pressure; 45 c.c. of fairly clear fluid was drawn and 15 c.c. of serum was injected. This fluid was sterile and gave a good reduction of Fehling's. For the next five days, the patient seemed to be doing well; his temp. was normal; he became clearer mentally; the retraction of the neck lessened, and he was able to take nourishment very well. On the 31st, however, a relapse occurred; the temp. went up to 103; the delirium returned. 45 c.c. of very purulent fluid was withdrawn and 35 c.c. of serum was injected. This fluid showed degenerated extracellular gram negative cocci by smear, but the culture was sterile. The punctures were repeated daily until August 10th; large amounts of fluid, 50 to 75 c.c. being withdrawn, but since the cultures were constantly sterile, small quantities of serum, 15 to 20 c.c. were injected. The fluids of the 5th, 6th, 8th and 9th showed a reduction of Fehling's. On the 6th, 9th, 13th and 16th, an autogenous vaccine was given in doses of 250, 500, 750 and 1,000 million; on the 11th, 13th and 16th, the punctures were repeated, the fluid becoming clearer and showing a good reduction of Fehling's and the quantity obtained by the last puncture being only 30 c.c. On the 19th, the condition was so much improved that a puncture was not considered necessary. Twenty punctures had been made. Much of the time the patient was desperately ill, the family gave up hope almost entirely and considerable persuasion was necessary to induce them to allow the treatment to be continued. The patient was extremely emaciated as most of the time during the relapse swallowing had been very difficult owing to the extreme retraction of the head. However, the recovery went on smoothly, though slowly. The absence of fever was very unusual, the temperature being elevated for only a day or two at the onset and again at the beginning of the relapse. Curiously enough when the delirium had entirely cleared up, he remembered nothing of his illness, nor of the punctures, simply that he had gone to bed with a very severe headache.

The other two cases were somewhat similar and need not be given in so much detail.

Case II.—W. P., young man, 20 years old; seen March 24th; pulmonary tuberculosis in an inactive form for two years; had not felt well for two weeks, but had been acutely ill only two days; the symptoms were typical of meningitis, and on lumbar puncture, 60 c.c. of cloudy fluid was withdrawn and 20 c.c. of antimeningitis serum was injected. The fluid showed meningococci both by smear and culture; Fehling's was not reduced. On March 25th, the temp. had fallen from 104 to 100.5 and the patient's condition was better; 75 c.c. of clearer fluid was withdrawn and 20 c.c. of serum was injected. This fluid showed meningococci by smear but not by culture and Fehling's was not reduced. On the 26th, temp. was normal, but punctures were repeated and serum was given on that and the following day, the fluid in both instances being sterile, and on the 27th reducing Fehling's. The patient continued to improve until the 30th when the temp. arose to 102.2 and there was severe headache. 50 c.c. of cloudy fluid was withdrawn and 20 c.c. of serum was given. This fluid was sterile; Fehling's was slightly reduced. The punctures were repeated on the 31st of March and on April 1st, 2d and 3d, the fluid of April 1st, only, showing meningococci; that of the 3d gave a good reduction of Fehling's. The temp. was normal on the 2d; the patient continued to improve until the 12th when he had a second relapse; temp. 101.5; severe headache; lumbar puncture 60 c.c. of cloudy fluid; 20 c.c. of serum. This fluid showed extra and intracellular meningococci, but the culture was sterile; Fehling's was not reduced. By this time the family was quite worn out by the care of the patient and he was taken to the Post Graduate Hospital where he recovered under serum treatment from the second relapse and has been well ever since. A second relapse is very unusual.

Case III.—W. R., 13 years old; seen July 26th; taken sick July 24th; symptoms characteristic; Herpes on lips and delirium; 50 c.c. of cloudy fluid showing meningococci by smear and culture; Fehling's not reduced, 20 c.c. of serum given. Lumbar punctures were done daily until August 6th, except on July 30th, August 3d and August 5th, when improvement clinically and in fluid had seemed to warrant their omission. At no time, however, had the temp. been normal or the patient's general condition very encouraging. On the 6th, the patient suffered considerably from shock at time of puncture and no more were attempted until the 12th. At this time the patient was greatly emaciated; the head was retracted and swallowing was almost impossible; temp. was 104; 50 c.c. of turbid fluid was withdrawn and 10 c.c. of serum injected without untoward symptoms; a small quantity was given on account of the shock at the previous injection. This fluid showed meningococci by smear and culture and no reduction of Fehling's; punctures were repeated on the 13th, 14th, 15th, 16th, 17th, 19th and 21st, no serum being given at the last puncture. Seventeen punctures were done. There were no organisms after the 15th and there was a good reduction of Fehling's after the 16th. An autogenous vaccine was given on the 13th, 17th and 21st in doses of 250,500 and 750 million.

Autogenous vaccines have not been used in a sufficient number of cases to show their value definitely. However, it is the custom of the Department to use them in cases that tend to become chronic and in a number of instances they seem to have been beneficial. In Case II, vaccine was not used, as both the first attack and the relapse cleared up promptly. It has been the experience of the Department that doses of serum larger than 20 c.c. need to be given with extreme caution even though very large amounts of fluid are withdrawn. It is to be remembered that relief of pressure is in itself of considerable therapuetic value. Untoward effects from the withdrawal of large quantities of fluid have never been observed. Of course, the punctures are always done with the patient lying down. The serum treatment should be continued until the fluid has been sterile for two or three days and until the patient clinically is much improved. In Case I, for instance, no cultures grew during the relapse, but eleven punctures were done, followed in all cases but the last one or two by the administration of serum.

It will have been noticed in the above reports that considerable prognostic importance is attached to the reduction of Fehling's. This has already been referred to in a " Summary of Four Years of Clinical and Bacteriologic Experience with Meningitis in New York City," DuBois and Neal, American Journal Diseases of Children, January, 1915, and " Streptococcus Meningitis, with Report of Cured Case," DuBois and Neal, Archives of Pediatrics, January, 1915. A more extended study of this point is now being made. In addition to the specific serum and vaccine treatment, in which one must be guided by experience, the general treatment is of great importance. Urotropin is given as a routine. It may or it may not be excreted as formaldehyde in the spinal fluid. It certainly tends to prevent cystitis if retention develops as it often does. Sedatives must be used if the patient is delirious or restless, preferably some preparation of opium, as it has a less depressing effect on the heart than other sedatives. The eyes, mouth, skin, bladder and bowels must all receive careful attention. Most important of all is the feeding. In many instances swallowing is very difficult on account of the retraction of the head and the greatest patience must be exercised to induce the patient to take even a little nourishment. Feeding by rectum was resorted to for a while in Case I. It is not, however, the object of this paper to cover fully the treatment of meningitis. Our experience in that direction has been dealt with more in detail in the summary mentioned above. The necessity of prolonged administration of the serum did, however, seem worth emphasizing.

My thanks are due to Dr. V. V. Bourke, Dr. B. Lefkovics and Dr. A. Corsiglia for permission to publish the above cases.

INTRASPINAL ADMINISTRATION OF ANTITOXIN IN TETANUS

NOTES ON A SERIES OF CASES*

MATTHIAS NICOLL, JR.

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As the result of a series of animal experiments conducted at the Research Laboratory of the Department of Health by Dr. William H. Park and myself[1] in order to determine the curative value of the intraspinal administration of tetanus antitoxin, and by which the superiority of this method was conclusively shown, every effort has been made during the past year to get into immediate touch with physicians and hospitals in and about the City of New York having cases of tetanus under their care, and induce them to give antitoxin as soon as possible by the following method:

1. From 3,000 to 5,000 units into the lumbar region of the spinal canal, preferably under an anesthetic, the volume of fluid injected being brought up to 10 or 15 c.c. by the addition of sterile normal saline, the exact amount being regulated according to the age of the patient and the amount of spinal fluid withdrawn.
2. Ten thousand units intravenously at the same time.
3. Repetition of the intraspinal dose in twenty-four hours.
4. A subcutaneous dose of 10,000 units three or four days later.

The well-recognized adjuvants to specific treatment—quiet, subdued light, sedatives, etc.—were, of course, understood as a necessary part of the therapeutic measures.

In no case has the physician objected to giving an intraspinal injection of antitoxin when so advised. Some, however, have given it in much greater quantities and by other methods in addition to that recommended, usually before I was able to consult with them.

Reports of twenty cases have thus been collected, in all probability representing a majority recognized as tetanus during this period in and about the City of New York. I have seen about half of the patients personally, in consultation. In others, the physicians have been kept in constant touch by telephone or through a clinical assistant during the progress of the case. In four cases, the histories have been furnished by the physician on request, the treatment given being exactly that recommended by the published article referred to above. In all cases the antitoxin used was furnished by the Department of Health.

A brief abstract of the clinical histories follows:

CASE 1.—F. D., girl, aged 10 years, seen in consultation with Drs. W. B. Anderton and A. A. Smith, fell, striking her forehead on the ground, receiving a lacerated wound three-quarters inch long over one brow. This was properly disinfected and sutured, healing promptly. Seven days later there was a facial paralysis on the side on which the wound was received. Thirty-six hours later the jaws were firmly locked. Eight hours after this symptom was noted, the patient received 3,000 units of antitoxin intraspinally and 10,000 intravenously. Several subcutaneous injections were later given. The tetanic spasms were largely confined to the muscles of the jaw and pharynx, and later, the abdominal muscles; attempts at swallowing and the slightest external irritation caused contraction of the muscles of the throat and larynx, cyanosis, general convulsions and unconsciousness. Such convulsions occurred on fifty or more occasions, together with innumerable minor spasms. Pneumonia developed later, resolution being very long delayed. After a protracted convalescence and extreme emaciation, the patient made a perfect recovery.

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* Read at the Meeting of the Association of American Physicians, Washington, D. C., May 11, 1915.

[1] Park, W. H., and Nicoll, Matthias, Jr.: Experiments on the Curative Value of the Intraspinal Administration of Tetanus Antitoxin, The Journal A. M. A., July 18, 1914, p. 235.

Case 2.—Thomas B., laborer, was admitted April 1, 1914, to the New York Hospital, with multiple lacerations of scalp and traumatic amputation of toes of the right foot. The wounds were immediately disinfected with iodin and irrigated with iodin solution. The following day, amputation of the toes was performed. April 10 (incubation nine days), there was slight stiffness of the jaws, which was not reported until the following morning. April 11, 1,500 units of antitoxin were given in the tissues about the wound and 3,500 intravenously, later on the same day, 3,000 units into the tissues about the wound and the same amount intravenously. April 12, the patient was very much worse, and was given 13,000 units intravenously, 8,000 intraneurally and 7,000 into the tissues about the wound. April 13, I was first consulted and the patient visited. His condition was still more unfavorable. There was marked opisthotonos. Eight thousand units of antitoxin were given into the spinal canal and 9,000 intravenously. Following the intraspinal injection the temperature rose to 105; there were severe headache, convulsions and semicoma. April 14, the patient was comatose throughout the day. April 15, the patient was conscious, and there was less rigidity. April 16, there was much less rigidity; the patient swallowed fairly well for the first time. The patient continued to improve and was discharged cured, April 30.

Comment.—Through a series of misunderstandings, this patient received still further intravenous injections of antitoxin following the intraspinal dosage, although an examination of his blood showed a tremendous antitoxic content. How much credit should be given the single intraspinal dose for the recovery in this case it is difficult to say. It is to be noted, however, that the first real improvement followed shortly after its administration.

Case 3.—Gustav H., aged 23, carpenter, admitted to St. Vincent's Hospital, June 27, 1914, had injured the tips of his fingers in some way, June 16. A definite history could not be obtained. Three days before this, the patient had a pustule on his neck, which was opened on the 20th. There was no other history of injury. June 21 (incubation five or eight days), he first noticed that his jaws were stiff; two days later, this was much more marked and had spread to the neck, back, arms and legs. On admission there was marked opisthotons, and the jaws were tightly locked. The slightest disturbance caused a convulsion. The patient's body rested on the heels and back of the head. The abdomen was boardlike. Treatment on the first day after admission consisted of 5,000 units of antitoxin intraspinally and 5,000 intravenously; June 28, 5,000 subcutaneously. The opisthotonos continued. July 1, 5,000 units were administered intraspinally, five hours after which the patient became delirious with a rise in temperature to 105. From July 4 to July 8, the rigidity decreased daily. July 18, the patient was discharged, cured.

Case 4—Boy, said to have been vaccinated sixteen days previously, the vaccination wound having apparently become infected one week after vaccination, developed a slight stiffness of the jaw. Four days after the onset of this stiffness, he was admitted to the Roosevelt Hospital, where it was noted that the legs were very stiff, the arms less markedly so. There was slight trismus. He was given, on admission, 2,500 units intraspinally, 5,000 units intravenously and 1,500 units intramuscularly and in the tissues about the wound. The latter two injections were repeated the next day. On the third and fifth day he received intraspinal injections. Improvement in symptoms began about the third day and continued until discharge thirteen days after admission, when patient was well.

Comment.—This case, though comparatively mild, was unquestionably one of tetanus. Many of the injections given could well have been dispensed with.

Case 5.—G. T., boy, aged 10 years, was admitted to New York Hospital, October 9, 1914. October 2, he ran a rusty nail into the sole of his left foot. October 9 (incubation seven days), there was stiffness of jaws, and some antitoxin (dose not known) was given intramuscularly. On admission, the teeth could not be separated and there was some tension in the calf of the left leg. All reflexes increased. Eight hours after admission, 3,000 units of tetanus antitoxin were given intraspinally and 1,500 about the wound, which had been opened. A few hours later, 1,000 were given intravenously. During the night, there were minor convulsions, accompanied by piercing screams every fifteen minutes. On the 12th, there were some dysphasia, abdominal rigidity and stiffness of the neck. The teeth were separated one-half inch. Intravenous injection of 9,000 units was made; there were general convulsions. October 11, there were convulsions lasting ten minutes, opisthotonos and a great deal of pain. October 12, there were convulsions for twelve minutes, stertorous breathing, cyanosis and great rigidity for half an hour following. October 13, the boy was comfortable and swallowed well. Intraspinal injection was made of 5,000 units. From October 14, there was a gradual improvement. The patient was discharged cured, October 24.

Case 6.—M. B., boy, aged 8 years; was admitted to Bellevue Hospital June 17, 1914. The history of injury due to a fall six days previously and the presence of an old ulcer on the right shin did not serve to establish the incubation of the disease. Five days before admission his jaws began to be stiff. This stiffness extended to other muscles day by day. On admission he was absolutely rigid from his neck to his feet. His teeth could be separated but one-fourth inch. June 18, under an anesthetic, he was given 5,000 units of antitoxin intraspinally and 5,000 subcutaneously. The mind was perfectly clear; the arms alone could be moved freely. There were incontinence of urine and feces. The boy was fed by gavage. June 25, he was very much improved. He bent at the waist when lifted. The mouth opened five-eighths inch. June 28, he moved the legs and arms freely; the spine was no longer stiff; the mouth opened widely; he ate well.

Comment.—This child was in a desperate condition at the time of giving antitoxin. On theoretical grounds we should not expect that antitoxin would be of much avail, when given at so late a period of the disease, and yet the improvement followed so soon after its administration that it would seem that antitoxin should be given the credit for it.

Case 7.—R. M., boy, was admitted to the Hospital of the Holy Family, April 15, 1914, having run a nail in the sole of his foot at some time previously not possible to determine. There was apparently locking of the jaws three days before admission, at which time the muscles of the neck and back were rigid. The wound on the sole of the foot was opened and dressed. April 16, 5,000 units of antitoxin were given intraspinally, 10,000 intravenously and 3,000 intramuscularly. The child's condition gradually improved, and he was discharged cured about two weeks after admission.

Case 8.—D. R., boy, aged 10 years, a patient of Dr. Ramsdell of White Plains, complained, September 14, 1914, of pain in the back and slight stiffness of the jaw and left arm. This child was vaccinated August 17. August 31, the vaccination wound not having been dressed for nine days, was in an unfavorable condition, there being considerable pus at the site of vaccination and swelling of the arm. A shield was later applied on the mother's initiative. There was a further history of injury to the wound on several occasions. The incubation of the disease could not, therefore, be determined. The symptoms of tetanus increased until September 18, when the patient was given an intraspinal injection of 5,000 units of antitoxin and 45,000 units (through a misunderstanding) intravenously. Following this there was a marked exaggeration of all the symptoms; a rise of temperature and opisthotonos, lasting about forty-eight hours. The patient gradually improved and was discharged cured in two weeks.

Case 9.—Boy, aged 6 years, a patient of Dr. Joseph Wheelwright, gave a history of trauma nine days before the locking of the jaws. Forty-eight hours later an intraspinal injection of 5,000 units and intravenous injection of 10,000 units of antitoxin were given. Subsequently this was repeated. The case was a very severe one with many convulsions, and the patient recovered fully in the fourth week.

Case 10.—A., male, at St. Vincent's Hospital, following a traumatic amputation of finger fourteen days previously, developed severe spasms of the abdominal muscles; later his jaw became locked and the rigidity was general; there were a number of convulsions. Thirty-six hours after the development of symptoms, 5,000 units of antitoxin were given intraspinally and 10,000 intravenously. There was marked anaphylaxis immediately following the injection, with urticaria covering the entire body and suggestion of edema of the glottis and lungs. This passed away shortly after the administration of a large dose of epinephrin. The intraspinal dose was repeated forty-eight hours afterward, as the patient had grown worse and had had a number of convulsions. The serum reaction following this was not so severe as in the first instance. Improvement in symptoms began to show in two or three days, the patient making a perfect recovery from his tetanus, but developed mental symptoms consisting of intermittent attacks of acute mania and delusions of persecution. After a number of weeks this completely disappeared and his condition was normal.

Case 11.—T. D., woman, aged 30 years, was seen by Drs. William H. Ross and H. E. Chauvin of Brentwood, N. Y., September 30, 1914. She had given birth to a child two weeks previously in very dirty surroundings. The symptoms of tetanus were very marked, and many convulsions continued for five days. Two days after the onset of symptoms, 10,000 units of antitoxin were given intraspinally and 5,000 intravenously; the later dose was repeated three times in three successive days. The patient was discharged cured except for a slight trismus which lasted for three days longer, October 11.

Case 12.—R. W., boy, aged 11 years, was seen by the physicians mentioned above, September 6, 1914. There was a wound over one patella, received eleven days previously. Five thousand units of antitoxin were given intraspinally and 5,000 intravenously on the day following the development of symptoms. The intravenous dose was repeated. Eight convulsions occurred during the first three days, the temperature running high during this time. . The patient was discharged cured eight days after admission.

Case 13.—W. M., man, aged 20, seen by the same physicians January 3, 1915, had received a punctured wound of the instep three weeks previously. There was complete trismus and stiffness of the whole body. Ten thousand units of antitoxin were given intraspinally and 5,000 intravenously the day after the symptoms were noted. The intravenous injection was repeated the next day. The patient was discharged cured in two weeks.

Case 14.—M. G., woman, aged 22, gave no history of traumatism. Four days before admission to the Lebanon Hospital, there was slight rigidity of the neck and difficulty in opening the mouth. The next day the jaws were completely locked. On the day before admission she had general convulsions. On the day following admission, five days after the onset of symptoms, she was given 5,000 units of antitoxin intravenously and 750 units intraspinally. On the second and third days she was given 5,000 units intraspinally under an anesthetic. The patient also received injections of magnesium sulphate (25 per cent.) subcutaneously and intramuscularly three times a day in doses of 30 c.c. for seventeen days, without apparent relaxing effect. The course of the disease was rather protracted with many convulsions. The patient was discharged cured seven weeks after admission.

Case 15.—L. M., male, aged 18 years, was admitted to St. Peter's Hospital, March 27, 1915, with a crushed and lacerated wound of the great toe. The wound was immediately disinfected and properly dressed. March 30, the tip of the toe sloughed off. April 6, the patient had a severe chill and complained of sore throat. There was noted a slight stiffness of the jaw. In the afternoon there were marked stiffness of the back, and occasional slight convulsions when the patient was disturbed. Twenty thousand units of tetanus antitoxin were given intraspinally four hours after the occurrence of the chill. On the 7th, the symptoms increased in severity, convulsions occurring on the slightest irritation. There were marked opisthotonos and rigidity. Ten thousand units of antitoxin were given intraspinally under an anesthetic. On the 8th, the patient's condition was about the same. Ten thousand units of antitoxin were given intravenously. After the tenth day the convulsions stopped; the rigidity continued some time longer. April 22, the patient was up and about with slight rigidity. He was discharged cured a few days later.

Case 16.—M. S., man, aged 24, admitted to Bellevue Hospital, February 4, 1915, with very indefinite history, was said to have run a splinter into his foot three weeks previously. Two days before admission he complained of great pain in his back and neck, which was stiff; later he could not open his mouth. On examination the day after admission, it was found that his jaw was markedly locked, the abdominal muscles of a boardlike rigidity, and the back slightly arched. He was given 5,000 units of antitoxin in the spinal canal and 10,000 in the vein. The intraspinal dose was repeated two days later. The symptoms increased in severity for a day or two, frequent tonic spasms causing the patient to cry out with pain. After a few days all symptoms began to ameliorate, and he was completely well in about two weeks.

Fatal Cases.

Case 17.—O. R., man, aged 34, was admitted to Bellevue Hospital, November 5, 1914, for a lacerated wound of the sole of the foot, which, although treated, became infected with a foul-smelling, purulent discharge. November 12, seven days later, the patient complained of difficulty in swallowing, and the jaws were stiff. He received 1,500 units of antitoxin intramuscularly, and twenty-four hours later, 10,000 units intraspinally and 10,000 intravenously. November 14, the temperature was lower, the jaws less stiff and the general condition improved, when the patient became markedly cyanotic, went into a condition of status epilepticus and died in twenty minutes. No necropsy was held. The exact reason for the sudden death of this patient, when the course of the disease had apparently been arrested, is by no means easy of explanation.

Case 18.—J. O., man, aged 32, admitted to St. Peter's Hospital, November 13, 1914, had stepped on a rusty nail, November 1. Seven days later, his jaw became stiff. This stiffness increased until the time of the admission, when his teeth could hardly be separated. The muscles of the back and neck were stiff. November 13, five days after the onset of symptoms, he received 6,000 units of antitoxin intraspinally and 5,000 intramuscularly, the next day, 5,000 intramuscularly and 5,000 intravenously, and on the following day, 5,000 units intravenously and 7,000 intramuscularly. The patient had tonic and clonic convulsions in great numbers throughout the course of his illness. On the day before his death, the tonicity of the neck, jaws and abdomen had greatly diminished. He died following a convulsion, November 17. The short incubation in this case and long delay before beginning treatment are sufficient reasons for the failure of the antitoxin to save this patient's life.

Case 19.—E. C., boy, aged 13 years, admitted to Bellevue Hospital, June 30, 1914, fourteen days previously had fallen and cut his head. Eleven days later he had abdominal pains and vomiting, which were regarded as due to appendicitis. On admission to the hospital he had marked opisthotonos, locked jaws and inability to swallow. A convulsion occurred on the slightest disturbance. He received 10,000 units of antitoxin intraspinally and 10,000 units intramuscularly. The patient's condition grew worse from the time of his admission and he died within twenty-four hours. The disease had evidently progressed too far to be influenced by antitoxin.

Case 20.—W. R., physician, aged 53, was admitted to the French Hospital for herniotomy, which was performed, April 25. May 3, the patient complained that his neck felt stiff; May 4, this symptom was more pronounced and there was some rigidity of the jaw. Toward evening he could not open his mouth fully. He was given about 9,000 units of antitoxin intramuscularly. May 5, he had a general convulsion and became quite cyanotic; this was repeated twice, convulsions lasting about ten minutes. Five thousand units of antitoxin were given intraspinally and 15,000 intravenously. May 6, the patient had four convulsions, one lasting fifteen minutes. Five thousand units of antitoxin were given intraspinally. May 7, there were no further convulsions. The patient was able to take fluids by mouth, and there was much less rigidity. At 8 o'clock in the evening he had a sudden attack of pulmonary edema and rise of temperature. Later there were signs of acute cardiac dilatation. He died on the morning of May 8. This patient was very stout, and had chronic bronchitis and recurring attacks of asthma.

In judging the effect of antitoxin given intraspinally in this series of cases, it must be remembered that the patients were not selected, but that every case of tetanus reported was given the benefit of the treatment regardless of the clinical condition. The series, therefore, may be said to be fairly representative of the type of the disease occurring in and about the City of New York. A few of these patients would undoubtedly have recovered if the intraspinal injection of antitoxin had not been given or, indeed, without any treatment other than symptomatic. The results obtained, however, in the saving of life are so much more favorable than those in previous years, when large doses of antitoxin were recommended to be given by the intravenous and subcutaneous methods, that there can be no reasonable doubt that the low death rate, 20 per cent., here obtained was largely due to intraspinal dosage.

I wish to express my thanks to the following physicians, in addition to those mentioned above, for allowing me the privilege of seeing the patients in their hospital service, furnishing me with the clinical histories and permitting publication of extracts from them: Drs. Charles L. Gibson, George Stewart, John S. Thatcher, La Fetra, Joseph B. Bissell, M. S. Kakels, Charles Nammack, and Frank Overton of the State Department of Health.

AN EPIDEMIC OF WHOOPING COUGH TREATED WITH PERTUSSIS STOCK VACCINE

Matthias Nicoll, Jr., and Paul Luttinger

In the early part of February, 1915, a group of children came down with whooping cough at the Angel Guardian Home, Brooklyn. The Department of Health, through the Division of Laboratories, was requested to cooperate with those in charge of the Institution in controlling the expected epidemic.

On the first visit (February 26), it was found that the sanitary conditions of the home were excellent. The inmates were divided into two principal groups, occupying separate buildings, known as the *Nursery* and the *Home,* the first containing children less than five years old and the second those ranging between five and seven years of age. There was also a small pavilion known as the *Quarantine.*

The Quarantine Group consisted of 8 children who had contracted pertussis at the beginning of February. Half of them came from the *Nursery* and the other from the *Home.* They had all been whooping or having a paroxysmal cough with vomiting for two or three weeks, and had been treated with small doses of a commercial vaccine. The children were getting worse and losing strength from lack of appetite and the vomiting of the little food taken.

The following table (No. I) summarizes the treatment and course in this group. It will be noted that the vaccine treatment had little if any effect on the length of the disease. The average duration of the paroxysmal stage being 40 days. Case No. 4, which contracted pneumonia and recovered, may have been influenced by the three injections of vaccine, but this is problematical.

The vaccine used in these cases, as well as all others, was prepared by Dr. Povitzky of the laboratory staff, by scraping off a 48-hour Bordet-Gengou growth (several recent strains), emulsifying it in physiological salt solution, by shaking for two successive days in the shaking apparatus and then heating at 56° C. for 90 minutes; the emulsion being left in the ice chest between the two shakings.

The Nursery Group—The *Nursery* contained three divisions, living respectively on the *ground floor,* the *first floor* and the *second floor.* The ground floor division had a subdivision known as the *Crib room.*

The *ground floor* contained 32 children, who were either whooping or on the point of beginning to whoop. These children had had no treatment whatever, and it was the intention to divide them into two equal classes, treat one of them and leave the other as controls, but the authorities did not approve of this plan and insisted on having the thirteen worst cases injected at our first visit and later prevailed upon us to treat some of the control cases who were developing severe symptoms.

Table No. II shows the course of the 17 cases treated with the vaccines. It can be seen at a glance that the duration of the paroxysmal stage in this group was about two weeks shorter than in Group I, the average length being 25 days.

Controls—The 15 control cases were not real controls, for the reason above stated, and as is also shown by the fact that 8 of the 15 "control" cases proved to have non-specific coughs, which subsided within one week; the ninth case (No. 36) proved to have had pertussis before. Two had a paroxysmal cough which subsided within two weeks (Nos. 37 and 29). Of the remaining four cases, which were the only actual whoopers, we were prevailed upon to treat two (Nos. 26 and 31), whose duration was 7 and 21 days respectively. The two untreated cases (Nos. 35 and 39) had a paroxysmal stage of 34 and 31 days respectively.

Crib room—This contained 15 babies in their cribs. The maid in charge stated that some of them were whooping. These were treated and about half of the others were immunized. As there was no reliable observation on these cases, no definite conclusions could be drawn. We never heard a paroxysmal cough in the room. The treated as well as untreated stopped coughing within two weeks. One six-month old girl, suffering from marasmus, died on the 12th day of an apparently mild attack of whooping cough. She had received two injections of vaccine.

The Home Group—Ten girls, mostly from the Kindergarten class, who had been exposed to the four cases subsequently isolated at Quarantine, developed coughs more or less paroxysmal in character. Only one had an actual whoop; while another had sub-conjunctional hemorrhages but no whoop. They all responded to the vaccine treatment and stopped coughing paroxysmally within three weeks. Some of them, undoubtedly, had simple bronchial coughs, and it is a question whether any besides the two cases cited had any paroxysms. It would be best, therefore, to draw no conclusions from the treatment of this group.

Immunization—We began the immunization of the children exposed, but showing no symptoms, on our second visit to the institution (March 1st, 1915).

There were 34 girls on the first floor of the Nursery who had been directly exposed to the disease. Eighteen of them received three prophylactic doses of 500, 1,000 and 1,000 million each at three days interval. The remaining 16 were left as controls.

Among the 18 immunized girls, 5 began coughing at the second injection and 2 refused to submit to further immunization. Four of these 7 stopped coughing within a week. The 3 others began to whoop and vomit on the day of the third prophylactic dose and were treated with vaccines curatively. The average duration of their paroxysmal stage was 16 days.

Of the non-immunized cases, 4 developed simple coughs within three days. Two of these subsided, while the others began whooping; the most severe case was treated with vaccines. The duration of the paroxysmal stage in this case was 21 days; the untreated, milder case only lasted 16 days.

Neither the immunized nor the non-immunized cases developed any symptoms later than four days from the date of the first injection and the experiment remains, therefore, inconclusive.

The second floor of the Nursery was occupied by 98 boys, who had not been in direct contact with the disease. Thirteen of them had had pertussis before. Forty-two of the remaining were immunized and so far have not shown any symptoms. Of the 43 non-immunized boys, one began to cough paroxysmally 9 days after segregation. The cough, without vomiting, lasted 12 days and was treated with three doses of vaccine. As there is some doubt as to the paroxysmal nature of the cough, and as this was an isolated case, it would be best to draw no conclusions whatever, even if these would seem to be in favor of immunization.

Finally, we immunized half of the 164 children at the Home, and thus far neither these nor the non-immunized children have shown any symptoms of whooping cough.

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TABLE I

Quarantine Group (All Females)

Serial No.	Case No.	Age (in Years)	Duration of Whoop or Paroxysmal Cough	Vomiting	1—Dosage in millions 2—Course	Treatment — Dates of Injections or Visits											Total Duration of Paroxysmal Stage	Remarks
						Feb. 26	Mar 1	Mar. 1	Mar 8	Mar. 11	Mar 15	Mar. 18	Mar. 22	Mar 29	April 6	April 12		
1	I	1	14 days	+	1	250	500	1,000	2,000	3,000	5,000	—	—	—	—	—	31 days	Epistaxis frequent
					2	—	Stationary	Improving	Improvement continues	Improvement continues	Stopped vomiting and whooping	Slight cough	—	Hardly coughs		Discharged		
2	2	6	11 days (parox cough)	—	1	250	500	1,000	2,000	2,500	—	—	—	—	—	—	31 days	Whooped only 14 days
					2	—	Whooped and vomited	Improving	Hardly whoops	Stopped vomiting	Stopped whooping	Discharged						
3	3	3 6, 12	14 days (parox. cough)	+	1	250	500	1,000	2,000	3,000	4,000	—	—		—		38 days	Sub-conjunctival hemorrhages Râles in both lungs —feverish
					2	—	Whoops	Worse	Stopped vomiting	Slow improvement	Worse	Eyes closed up	Stopped whooping, still vomits	Stopped vomiting		Discharged		
4	4	4	14 days	+	1	250	500	1,000	—	—	—	—	—	—	—	—	52 days	Broncho pneumonia
					2	—	Worse	Stationary	Temp. 104°	Temp. 101°	Worse	Stationary	Improving	Stopped whooping	Stopped coughing	Discharged		
5	5	5	14 days	+	1	250	500	1,000	2,000	3,000	5,000	—	—	—		—	54 days	
					2	—	Stationary	Stationary	Improving	Improving	Much improved	Improvement continues	Still whoops and vomits	Still whoops and vomits	Slight whoop	Stopped whooping		
6	6	7	14 days (parox cough)	+	1	500	1,000	2,000	—	2,500	—	—	—	—	—	—	31 days	Whooped only 12 days
					2	—	Whooping	Stopped vomiting	Slight whoop	Two whoops daily	Slight cough	Discharged						
7	7	7	14 days (parox. cough)	—	1	500	1,000	2,000	3,000	4,000	—	—	—		—	—	17 days	Whooped 29 days Râles both lungs
					2	—	Whooping and vomiting	Stationary	Improving	Slight whoop	Stopped whooping	Still whoops	—	Stopped whooping	Slight cough	Discharged		
8	8	6	14 days (parox cough)	—	1	250	500	1,000	2,000	2,500	5,000	—	—	—	—	—	36 days	Whooped 20 days Psoriasis of tongue Specific history Anemic
					2	—	Whooping and vomiting	Stationary	Improving	Stopped vomiting	Hardly whoops	Still whoops	Stopped whooping, Mar. 20	Discharged				

TABLE II

NURSERY GROUP (GROUND FLOOR, ALL FEMALES)

Serial No.	Case No.	Age (in Years)	Duration of Cough Before Whoop	Duration of Whoop or Paroxysmal Cough	Vomiting	1—Dosage in Millions 2—Course	Treatment Dates of Injections or Visits										Total Duration of Paroxysmal Stage (in days)	Remarks
							Feb. 26	Mar. 1	Mar. 4	Mar. 8	Mar 11	Mar 15	Mar. 18	Mar 22	Mar 29	April 6		
1	9	4 6/12	7 days	7 days	+	1	250	500	1,000	2,000	3,000	5,000	—	—	—		40	Bloody nasal discharge
						2	Temp 101°	Improving	Improving	Less night whoops	Stationary	Worse	Improving	Slight whoop	Slight whoop	Stopped whooping		
2	10	4 6/12	7 days	7 days	+	1	250	500	1,000	2,000	—		—	—	—		17	
						2	—	Worse	Improving	Stopped whooping, Mar. 5	Stopped coughing	Discharged	—	—		—		
3	11	4	7 days	.....	—	1	250	500	—	—	1,000	2,000	1,000	—	—	—	12	Stopped whooping Mar 13
						2	—	Whooping	Feverish and nervous	Feverish and nervous	Much improved	Stationary	Stationary		Whoops and vomits	Stationary		
4	12	3	7 days	.....	—	1	250	500	1,000	2,000	4,000	5,000		—	—		16	
						2	—	Whoops and vomits	Worse	No vomiting	Improvement continues	Stationary	Stopped whooping, Mar 16	Still coughs	Slight coughs	Slight cough		
5	13	4 6/12	7 days	.....	—	1	250	500	1,000	2,000	3,000	5,000	—		—	—	19	Stopped coughing April 12
						2	—	Whoops and vomits	Stationary	Stationary	Improving	Worse	Stationary	Stopped whooping, Mar. 19	Still coughs	Still coughs		
6	14	4 6/12	7 days	.....	+	1	250	500	1,000	2,000	2,500	—	5,000	—	—	—	15	Nasal discharge
						2	—	Whoops and vomits	Improving	Improving	No vomiting	Stopped whooping	Still coughs severely	Stationary	Slight cough	Stopped coughing		
7	15	5	7 days	.....	+	1	250	500	1,000	2,000	3,000	5,000	—		—	—	30	Bloody nasal discharge
						2	—	Whoops and vomits	Stationary	Stationary	Stationary	Stationary	Stopped vomiting	Slight whoop	Slight whoop	Stopped whooping, April 1		
8	16	4	7 days	.....	—	1	250	500	1,000	2,000	4,000	5,000	—	—	—	—	32	
						2	—	Whoops and vomits	Worse	Worse	Worse	Worse	Stationary	Stationary	Stationary	Stopped whooping, April 3		
9	17	4	7 days	7 days	—	1	250	500	1,000	2,000	2,500	5,000	—	—	—	—	18	
						2	—	Vomits	Stationary	Improving	Stopped whooping, Mar 9	Vomits	Stationary	Stationary	Stationary	Stopped coughing, April 8		
10	18	4	7 days	.....	—	1	250	500	1,000	2,000	3,000	5,000	—	—	—	—	30	Nasal discharge; few râles post.
						2	—	Whoops and vomits	Worse	Stationary	No vomiting	Vomits again	Much improved	Slight cough, no vomiting	Slight whoop	Stopped whooping, April 1		
11	19	4	7 days "croupy"	.....	—	1	250	500	1,000	2,000	3,000	5,000	—	—	—	—	30	
						2	—	Whoops and vomits	Worse	Improving	Much improved	Worse	Much improved	Slight whoop	No vomiting	Stopped whooping, Mar 30		
12	20	4	7 days	.....	—	1	250	500	1,000	2,000	—	—	—	—	—	—	14	
						2	—	Whoops and vomits	Improving	Worse	Feverish	Stopped whooping, Mar. 14	Slight cough	Slight cough	Slight cough	Stopped coughing		
13	21	3 6/12	7 days	7 days	+	1	250	500	1,000	2,000	3,000	5,000					42	Acute otitis media, stopped coughing, April 12
						2	—	Worse	Worse	Slight improvement	Improvement continues	Stationary	Much improvement	Slight whoop and vomiting	No vomiting	Stopped whooping, April 3		
14	22	4	7 days	3 days	+	1	500	1,000	2,000	3,000	5,000	—	—	—	—	—	11	
						2	—	Improving	Stationary	Stopped whooping, Mar. 9	Stationary	Stopped vomiting	Slight cough	No cough	Discharged			
15	23	4	7 days	1 day	+	1	500	—	—	1,500	3,000	—	—	—	—	—	15	
						2	—	Feverish and nervous	Feverish and nervous	Worse	Stopped whooping, Mar. 12	Slight cough	Slight cough	Slight cough	Stopped coughing	Discharged		
16	24	1	7 days	4 days	+	1	500	1,000	2,000	4,000	—	—	—	—	—	—	36	Bronchitis, stopped coughing April 6
						2	—	Worse	Stationary	Worse	Stationary	Improving	Slight whoop	Slight whoop	Slight vomiting	Stopped whooping, Mar 30		
17	25	5	7 days	7 days (parox. cough)	+	1	500	1,000	2,000	—	—	—	—	—	—	—	18	
						2	—	Stationary	Improving	Stopped parox cough	No vomiting	Stopped coughing	Discharged					

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EXPERIMENTS ON THE CURATIVE VALUE OF THE INTRASPINAL ADMINISTRATION OF TETANUS ANTITOXIN

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While tetanus antitoxin has proved most efficacious in the prevention of tetanus, its employment as a curative agent has been much less successful. This failure to produce more uniformly good results is chiefly due to its too late administration. Insufficient dosage and the use of the subcutaneous method have also been important factors. While the subcutaneous use of antitoxin, which at first was the usual method employed, may prove of value in large doses given within the first few hours after the onset of symptoms, nevertheless it frequently fails because of slow absorption and the time required to reach the tissues of the central nervous system. In the light of our present knowledge, this method can be justified only by an inability of the physician, for one reason or another, to give an intraspinal or an intravenous injection. The latter method is being more and more recommended, and when employed before the disease has made too much headway has given much better results than the subcutaneous.

From time to time since 1903, single cases or small series of cases have been reported in which the antitoxin has been given intraspinally. Thus Neugebauer[1] in 1905 reported forty-three cases from Continental and American sources with a mortality of twenty-two, or 51 per cent., and also three patients treated by him of whom two recovered. Many of these patients received also intravenous or subcutaneous injections or both. The results seemed to some observers to be better than when other methods were used without intraspinal dosage, but on the whole, this method of treatment has not made much headway. Experimental proof of the greater value of the intraspinal method has not until very recently been at all convincing. Permin of Copenhagen, working on rabbits and dogs, showed that local tetanus could be prevented by the simultaneous injection of tetanus toxin intramuscularly and a dose of antitoxin given in the spine, whereas with the same dose of antitoxin given intravenously, local tetanus occurred. Four hours after the giving of the toxin neither method of preventing the occurrence of local tetanus was efficacious, and when nine hours were allowed to elapse between the giving of toxin and antitoxin, the animals could not be saved.

The following series of experiments was undertaken with the object of determining to what extent tetanus antitoxin given in the spine has greater curative power, when the disease is actually established, than when given in the circulation. The comparative ineffectiveness of subcutaneous injections of tetanus antitoxin in developed cases is incidentally brought out. The use of guinea-pigs for this purpose has not been recorded so far as we know. They possess, however, a marked susceptibility to tetanus together with comparative freedom from intercurrent diseases and a certain hardihood in caring for themselves, feeding, etc., even when disabled, not shown by rabbits and other laboratory animals. The one great disadvantage to their use is the extremely small caliber of the spinal canal, for which reason a good deal of experimentation was necessary in order to perfect a technic of intraspinal dosage. Before undertaking the use of antitoxin by this method it was demonstrated by one of us (Nicoll) on dead and living guinea-pigs, that from 0.25 to 0.5 c. c. of a solution of anilin dye introduced into the spinal canal in the lumbar region rapidly finds its way up along the cord and even into the ventricles of the brain. It was then necessary to fix the effective limits of antitoxin given in the circulation as regards both the time elapsed after the giving of toxin and the dosage of the latter, and then to determine whether these limits were equaled or exceeded by intraspinal injection. It is well to emphasize at this time the fact, both as regards experimentally produced tetanus and the human form of the disease, that a very short delay in an overwhelming infection or too long a delay in any case before beginning treatment renders the action of antitoxin, by whatever method given, valueless as a curative agent.

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[1] Neugebauer: Wiener klin Wchnschr., 1905, xviii, 450.

It was found that two minimal fatal doses for a 350-gm. guinea-pig of a solution of dried standard toxin and the institution of treatment after the lapse of from seventeen to twenty-four hours afforded a good opportunity to contrast the value of different methods of giving antitoxin in animals showing the symptoms of tetanus.

Details of Experiments

Technic of Spinal Injection.—After the hair was clipped, a small transverse incision was made down to the spine at the level of the crest of the ilium. The spine was then sharply flexed by an assistant while the operator introduced a small hypodermic needle between two spinous processes in the middle line, the needle being directed from above downward and almost parallel with the spinal column. Injections were made slowly and the fluid warmed. It is not advisable to give a greater volume than 0.5 c. c. at one time. Paralysis occasionally follows the operation, but usually of a temporary character. Injections into the circulation were made into the heart. The latter operation after a little experience is easily performed and produces no ill effect on the animal.

TABLE 1

Administration and Result of Antitoxin in Experiment 1*

Number	Weight Grams	Method	Result
223	320	Control	D 4 days
956	290	Control	D 4 days
283	330	Subq.	D 4 days
263	330	Subq.	D 17 days
227	290	H.	Discharged
209	300	H.	D 7 days
251	305	H.	D 10 days
280	315	Sp.	Discharged
33	330	Sp.	Discharged
52	370	Sp.	Discharged
208	385	Sp.	D 4 days

* In this and the following tables, D = Dead number of days after receiving toxin; Subq. = Subcutaneously; H = Intracardially; Sp = Intraspinally; N = Intraneurally.

Experiment 1—December 1, two fatal doses of toxin for a 350-gm. pig were injected deeply into one hind leg, no allowance being made for body-weight. Twenty hours later all pigs showed more or less stiffness in the inoculated leg. Each was given 250 units of antitoxin by the methods designated in Table 1.

The death of Guinea-Pig 208 on the same day as the controls is in all probability to be accounted for by the faultiness of our early technic, the antitoxin probably not actually entering the spinal canal. The irregularity of the effect of the same dose of toxin on two pigs of the same weight is well shown in Guinea-Pigs 283 and 263. In the later experiments the degree of involvement of the inoculated leg at the time of giving antitoxin was carefully noted, as this factor was found to be of more importance in arriving at a prognosis than that of elapsed time alone.

Experiment 2—January 26, two fatal doses of toxin for a 350-gm. pig were injected deeply into one hind leg, no allowance being made for body-weight. Nineteen hours later all pigs showed more or less stiffness in the inoculated leg, and were given antitoxin in the amounts and by the methods designated in Table 2.

TABLE 2

Administration and Result of Antitoxin in Experiment 2

Number	Weight Grams	Amount Units	Method	Result
879	265	500	Subq.	D 3 days
198	305	500	Subq.	D 5 days
48	320	500	Subq.	D 3 days
664	405	500	Subq.	D 4 days
878	290	500	H.	D 8 days
211	315	500	H.	D 3 days
491	355	250	H.	D 7 days
876	410	250	H.	Discharged Feb. 20
616	310	250	Sp.	D 7 days
580	350	250	Sp.	Discharged Feb. 20
875	360	250	Sp.	Discharged Feb. 20
874	375	250	Sp.	Discharged Feb. 20

This brings out well not only the superiority of the intraspinal method, but also the great inferiority of the subcutaneous way. The one surviving intracardial case, Guinea-Pig 876, being over the standard weight, received rather less than two fatal doses of toxin.

Experiment 3—March 12, seven pigs were injected deeply into one hind leg with two fatal doses of toxin. The amount was proportioned to body-weight of each. Nineteen hours later they were given antitoxin in the amounts and by the methods designated in Table 3, the inoculated leg being stiff in the degree noted at the time.

TABLE 3

Administration and Result of Antitoxin in Experiment 3

Number	Weight Grams	Cond. of Leg	Method	Amount Units	Result
488	250	Stiff	Control	...	D 2 days
442	295	Slightly stiff	Control	...	D 3 days
499	350	Slightly stiff	Control	...	D 3 days
480	275	Slightly stiff	H.	100	D 13 days
498	275	Stiff	H.	200	Discharged April 10; drags leg
451	265	Fairly stiff	Sp.	10	Discharged well April 23; drags leg
479	270	Stiff	Sp.	50	Discharged well April 10; drags leg

It is to be noted that each pig received exactly two fatal doses of toxin and that the degree of tetanic spasm in the inoculated leg was carefully observed at the time of giving antitoxin. The one surviving intracardial case received twenty times the amount of antitoxin of one intraspinal case and four times the amount of the other.

Experiment 4—March 24, eighteen pigs were injected into the hind leg with two minimal fatal doses of toxin proportioned to the body-weight of each. Twelve of these were given antitoxin in the amount and by the methods indicated, seventeen and one-half to eighteen hours later, the inoculated leg being stiff in the degree noted at the time. The remaining six were held for twenty-two and one-half to twenty-three hours after inoculation, as the stiffness was not marked until that time.

TABLE 4

Administration and Result of Antitoxin in Experiment 4

Number	Weight Grams	Cond. of Leg	Method	Amount Units	Result
116	290	Fairly stiff	Control	...	D 3 days
42	310	Fairly stiff	Control	...	D 3 days
296	250	Slightly stiff	H.	100	D 8 days
227	275	Fairly stiff	H.	100	D 4 days
399	300	Fairly stiff	H.	100	D 5 days
316	255	Slightly stiff	N.	200	D 4 days
287	255	Fairly stiff	N.	200	D 3 days
289	280	Fairly stiff	N.	200	D 3 days
306	285	Slightly stiff	N.	200	D 3 days
59	255	Stiff	Sp.	10	Discharged April 23; normal
304	275	Fairly stiff	Sp.	10	Discharged April 23; drags leg
321	320	Fairly stiff	Sp.	10	Discharged April 23; drags leg

Six Given Antitoxin 22½ to 23 Hours After Inoculation

102	300	Stiff	H.	100	D 5 days
10	325	Stiff	H.	200	D 4 days
272	350	Stiff	H.	200	D 4 days
263	285	Stiff	Sp.	50	Discharged April 23; drags leg
123	325	Stiff	Sp.	50	D 5 days
294	350	Stiff	Sp.	50	Discharged April 23: drags leg

This would seem absolutely conclusive of the superiority of the intraspinal method of giving antitoxin over the intravenous method. It will be noted that only those animals receiving the antitoxin in the spine were able to survive. This result is the more striking since the amount of antitoxin given them was only a fractional part of that given in the circulation. An attempt was made to give the antitoxin to four guinea-pigs intraneurally. Under an anesthetic the sciatic nerve of the affected limb was cut down on and freed from the surrounding tissues, as much antitoxin as could be introduced into the nerve sheath injected, and the remainder intramuscularly directly along the course of the nerve. Owing to the small caliber of the nerve, most of the antitoxin passed to the deep intramuscular tissues. The animals lived no longer than those receiving subcutaneous injections.

In all of these experiments no guinea-pigs were discharged from observation until there was absolutely satisfactory evidence that the disease had long ceased to make any progress. While a number of the animals, perhaps most, on being discharged exhibited more or less stiffness in the inoculated leg lasting for weeks or even months, they were nevertheless in the best of health in other respects. This condition appears to be identical with that seen in human tetanus, in which the stiffness in different groups of muscles is often very prolonged even when the patients are perfectly convalescent.

Since beginning this work, we have obtained records of four consecutive clinical cases of tetanus in which an intraspinal injection of antitoxin was given and all the patients recovered. Two of these occurred in private practice and two in hospitals. In all of them, in addition to the antitoxin used intraspinally, very much larger amounts were also given by other methods.

Case 1—Girl, aged 10 years. Incubation of the disease, nine days or less. Cephalic type, of great severity, with many convulsions. Six hours after the locking of the jaws, 3,000 units of antitoxin were given in the spine and 10,000 in the vein. A facial paralysis had been present, however, on the side of the head on which the wound had been received for thirty-six hours previously. In all, 52,000 units were given, with the exception of the first dose, intravenously or subcutaneously. The blood showed four units of antitoxin to the cubic centimeter at the time of the last injection, about one week from the onset of the disease.

Case 2—Adult, male. Incubation nine days. Not given treatment for twelve hours or more after stiffness of jaws was noted, and then 1,500 units of antitoxin were given in the tissues about the wound and intraneurally, 3,500 intravenously and 300 subcutaneously; the next day 13,000 intravenously, 8,000 intraneurally and 7,000 subcutaneously. On the third day, the patient continued to grow worse with increasing rigidity, and was given 9,000 units intravenously, 8,000 intraspinally and 7,000 units into the tissues of the wounded foot. Two days later, there was noted less rigidity and the patient was able to swallow and separate the jaws to a greater degree. On this day 7,500 units of antitoxin were given in the vein, and on the following day 5,000. The blood showed 6 units of antitoxin to the cubic centimeter at a time just previous to the final injection. The patient was discharged cured after an illness of extreme severity, twenty days after the onset of symptoms. The intraspinal injection having been given so late in the disease cannot logically receive the credit for the patient's recovery, and yet the first improvement in symptoms was noted the day following the dosage given in this way.

Case 3—Boy, aged 9 years. Incubation not obtainable, but not over two weeks. Symptoms present for fully two days before treatment, when he received 5,000 units of antitoxin intraspinally, 3,000 subcutaneously and 10,000 intravenously, and on the next day 5,000 units in the spine. This case, while not so severe as the others, was well marked and typical of the disease.

Case 4—Child, aged 6 years. Incubation ten days, 3,000 units of antitoxin in the spine in the afternoon of the day on which symptoms were noted. At the same time 1,300 units were given in the veins. All subsequent dosage was intramuscular. The case was of a severe type with many convulsions.

It may be added that during this time we have had a record of another case of great severity, incubation twelve days, in which recovery followed large doses of antitoxin given principally in the vein but also in the tissues. No intraspinal dosage was given.

While this series of four cases is entirely too small to justify the conclusions that the giving of antitoxin intraspinally was the factor which determined the favorable outcome of the disease, especially as a much greater amount of antitoxin was also administered by other methods, the results are, nevertheless, in marked contrast to the average of those obtained in a much greater number of cases after the use of antitoxin in large doses given other than intraspinally.

Permin,[2] after rendering the blood of rabbits and other animals strongly antitoxic by the intravenous injection of antitoxin, was able, nevertheless, by introducing tetanus toxin by way of the nerves, to produce a local form of the disease, from which he reasons conversely that tetanus toxin, having once reached the spinal centers, cannot be dislodged by antitoxin given by the route of the blood, and therefore, all methods of giving it for curative purposes except that of direct application to the spinal cord can have little or no effect. Now it may be conceded that when the toxin has become firmly bound to the central nervous system, dissociation by the use of antitoxin is not possible. Nevertheless, one can never know in a given case how firm this union has become, and the statement that intravenous or subcutaneous injection is useless seems to us of too sweeping a character, especially since it will be noted that in the foregoing experiments a number of guinea-pigs in which the symptoms, when treatment was given, showed every evidence of the toxin having reached the spinal nerve centers, nevertheless recovered after a large dose of antitoxin given in the blood. Furthermore, we have one case of recovery in a guinea-pig with marked symptoms of tetanus following a very large subcutaneous dose of antitoxin.

On experimental and clinical grounds the following recommendation for the treatment of tetanus with antitoxin would seem to be amply justified: In every case strongly suspected of being tetanus, from three to five thousand units of tetanus antitoxin should be given at the first possible moment intraspinally, slowly by gravity, and always, if possible, under an anesthetic. In order to insure its thorough dissemination throughout the spinal meninges the antitoxin should be diluted if necessary, to a volume of from 3 to 10 c. c. or more, according to the patient's age. When fluid is drawn off previously to the giving of the antitoxin, an amount of the latter somewhat less than that of the fluid withdrawn should be given. A number of cases of " dry tap " have been observed in the disease by those so expert in spinal puncture as to leave no room for doubt that the canal was properly entered. In such cases only a small amount of tetanus antitoxin should be injected (from 3 to 5 c. c.). In brief, tetanus antitoxin should be used in precisely the same way as antimeningitis serum.

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[2] Permin, Carl: Mitt. a. d. Grenzgebiet. d. Med. und Chir., 1913, xxvii, 1.

Unlike experimental tetanus it must be remembered that in the human type of the disease there is frequently a focus constantly pouring out more and more toxin, for which reason it is probably advisable to repeat the intraspinal injection in twenty-four hours. While unquestionably the blood will soon become antitoxic through this intraspinal use of antitoxin alone, yet in order to insure the quickest possible neutralization of all toxins in the tissue-fluids, it would seem advisable to give at the same time, a dose of 10,000 or 15,000 units of antitoxin in the vein. A similar dose given subcutaneously three or four days later will insure a continuance of the highly antitoxic condition during the next five days. We do not believe there is any advantage in giving larger amounts of antitoxin than those indicated.

The proper and thorough surgical treatment of the wound and the use of sedatives, stimulants, etc., are very essential factors in the treatment of the disease· Finally, intraneural injections of antitoxin seem to us to be an uncertain and roundabout way of reaching the diseased nerve-centers, which may be much more simply and thoroughly accomplished by the simpler procedure of intraspinal injections.

When for various reasons tetanus antitoxin cannot be given in the spinal canal, it is far better to inject it at the earliest possible moment intravenously than to lose time in seeking skilled advice in order to give it intraspinally, which, however, should be done later. If, which is hardly conceivable, a physician is unable to give it intravenously, he should give it subcutaneously. This, though far inferior than even the intravenous way, is still of some value in early and slowly developing cases.

Note.—We have at present (July 8) two additional patients under observation each of whom is convalescent from a severe attack of tetanus. The incubation of the disease could not be obtained in either case. The antitoxin was given by the exact method and in the amounts recommended above.

ACTIVE IMMUNIZATION IN DIPHTHERIA AND TREATMENT BY TOXIN-ANTITOXIN

William H. Park and Abraham Zingher

Assisted by

Harry M. Serota

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Part I

The property of toxin-antitoxin mixtures to produce immunity in animals has long been known. One of us (W. H. P.) published a series of successful results in 1903. Smith suggested the attempt to immunize children, but no one undertook it until 1912, when von Behring made the attempt.

In May, 1913, v.Behring published the results obtained in human beings. The combined substances were either neutral or slightly toxic to the guinea-pig. Individuals were injected with small doses (from 1/20 to 1/10 c.c.) which were repeated in from seven to ten days. The first injections were made subcutaneously or intramuscularly, while later the intracutaneous administration was favored, since the more distinct local reaction was believed to induce a greater efficiency of vaccination.

The results as reported by different observers have not been uniform and as a rule are not distinctly stated. Hahn reports antitoxin production in thirty-six out of forty treated; as we shall see later, the fact that twenty-five of the forty patients had natural antitoxin already present modifies greatly the favorable nature of this result. Failure to respond was at first attributed to a lowered general condition, but now we realize that it was because the susceptibility to the immunizing response to toxin-antitoxin varies. It seems greatest in young adults. Zangmeister reports the interesting fact that the new-born are nearly a hundred times less susceptible to vaccine than adults. Kleinschmidt and Viereck report the active immunization of twenty-seven cases, but give no definite data. They advise combined active and passive immunization in persons exposed to diphtheria and also suggest the use of the Schick reaction in determining the antitoxin content of persons after vaccination. Kissling reports results at Hamburg-Eppendorf in the immunization of 310 patients who were exposed to infection. In the 111 who were injected twice, no diphtheria developed. In the 199 cases injected once, 8 patients developed clinical diphtheria. During an epidemic of diphtheria, Hahn and Sommer vaccinated part of the inhabitants of several villages: of 633 patients considered fully immunized only two developed mild diphtheria, but 10 of the group developed it during the first ten days. The disease continued to spread to some extent among those not vaccinated. Bauer finds that the persistence of the bacilli in carriers is not shortened by active immunization. He believes that all patients without natural antitoxin can be immunized, if sufficient doses and amounts of toxin-antitoxin are injected.

The interval between the injections of toxin-antitoxin and development of antitoxin varies in different cases. According to Hahn it is usually not less than three weeks. Kleinschmidt and Viereck found it shorter—even eight days. Patients with natural antitoxin usually show early and considerable antitoxin production. They also generally show a somewhat greater local susceptibility to toxin-antitoxin mixtures. This according to von

Behring is due to sensitization from previous infections with the Klebs-Loeffler bacillus. This explanation we consider extremely improbable.

According to von Behring, the higher temperature of fever will destroy antitoxin at an increased rate, so that an amount of antitoxin, which generally protects, fails to do so in measles and scarlet fever. Schick and Karasawa find, however, that no change in antitoxin content is caused by measles. Von Behring considers that 0.01 unit per cubic centimeter is sufficient to protect healthy persons. Less than this probably suffices except under unusual conditions, as in scarlet fever.

PERSONAL EXPERIENCE

During the past twelve months the attempt has been made to immunize actively against diphtheria the patients in the scarlet fever wards of the Willard Parker Hospital. Owing to the large number of diphtheria-carriers admitted, the conditions in this hospital during the winter were such that on the average about one-quarter of the inmates were diphtheria-carriers.

For this purpose mixtures of diphtheria toxin and antitoxin were prepared, either slightly antitoxic, neutral or slightly toxic to the guinea-pig. A strong diphtheria toxin was used, where the minimum lethal dose was 0.0023 c.c. and L + dose 0.27 c.c. The mixtures represented B = 50 per cent., A = 66 per cent., G = 66 per cent., F = 80 per cent. and E = 90 per cent. of the L + dose of toxin to each unit of antitoxin. The L + dose of toxin is the amount which, when mixed with 1 unit of antitoxin and injected into a 250 gm. guinea-pig, will cause its death at the end of four days. One c.c. of Mixture E caused when injected into a guinea-pig at first a slight local induration and then in about twenty days paralysis.

The injections were made subcutaneously or intramuscularly in doses of from 0.25 to 1 c.c. of undiluted vaccine; a few of the non-immune persons received as high as from 3 to 5 c.c. at each injection. The dose was repeated two or three times at intervals of from three to seven days. The injections were made posteriorly in the inter-scapular region, in some over the insertion of the deltoid.

The local reactions consisted as a rule of varying degrees of redness, induration, pain and tenderness; in part they seemed to depend on the size of the dose and in part on the individual susceptibility. Persons with natural antitoxin gave somewhat more frequently the stronger local reactions. The constitutional symptoms were as a rule rather mild; occasional temperature reactions of from 1 to 3 degrees were noted after larger doses of Mixture E.

The results of the active immunizations were controlled by determining the antitoxin content before the injection; again three weeks and more after the injections similiar examinations were made. The antitoxin content of the sera was estimated by a modification of Römer's intracutaneous method on the guinea-pig. As it was not possible to detect less than 1/160 unit in 1 c.c. of serum, anything less than that was considered as absence of antitoxin, although naturally some sera not showing this amount may have had a slight trace.

A group of 68 patients was injected with the various mixtures of toxin-antitoxin; of these, 44 had, before the injections, sufficient natural antitoxin to protect them against diphtheria. They all showed a decided production of antitoxin in a relatively short time (in some as early as seven days). The remaining 24 patients had no appreciable antitoxin before the injections; of these 6 gave rise to distinct antitoxin production, while 18 failed to respond.

Five patients of the 18 which did not respond developed slight or moderate clinical diphtheria two weeks or more after the first injection. Four had tonsillar exudates and one nasal diphtheria with visible membrane in the anterior nares. Cultures were positive, and the isolated Klebs-Loeffler organisms virulent.

We thus found that persons with natural antitoxin gave a ready response to active immunization, while those with no appreciable natural antitoxin failed to do so in a considerable proportion of cases. A larger group of susceptible patients was, therefore, selected to determine these results in a greater number of cases. For this purpose the intracutaneous reaction of Schick with diluted diphtheria toxin was used. This reaction depends on the local irritant action of toxin in the absence of antitoxin. A dilution of diphtheria toxin is made of such strength that 0.1 c.c. contains 1/50 minimum lethal dose for the guinea-pig. This amount is injected intracutaneously on the flexor surface of the forearm. A positive reaction appears in from twenty-four to forty-eight hours, and is characterized by a circumscribed area of redness and slight infiltration, which remains for from seven to ten days. After fading, a brownish pigmented area is left for days and weeks which in beginning generally shows superficial scaling. According to Schick, a positive reaction indicates that there is less than 1/30 unit of antitoxin in 1 c.c. of blood. The majority have none at all. Such persons he considers susceptible to diphtheria. Our estimations agree with his conclusion.

The Schick reaction was again employed three weeks and more after the vaccination to indicate clinically whether or not the patients had responded to the immunization. In many, bleedings were taken and tested in addition before and after the injections.

Ninety persons without appreciable antitoxin were thus selected who received full immunizing doses of mixtures E and F; of these only twenty, or 22 per cent., gave rise to a decided production of antitoxin (1/20 up to 10 units per cubic centimeter). Among the remaining seventy patients a considerable number were found in whom the Schick reaction was fainter, but not obliterated when the tests were made four weeks after the injections; the antitoxin produced, however, was too small in amount to be detected in the animal test. Seventeen of the seventy patients who failed to respond developed clinical diphtheria twelve days or more after the first injection of the toxin-antitoxin; those who showed symptoms before twelve days were not included in the group. Twelve patients had purulent nasal discharges sometimes tinged with blood, four tonsillar exudate, and one laryngeal diphtheria. The cultures were positive, and the isolated organisms virulent.

We note that active immunization in this group of susceptible persons gave rise to decided antitoxin production in 22 per cent. of cases; the interval between vaccination and development of immunity was generally long, as a rule not less than two weeks. Under conditions of exposure about 20 per cent. of those who failed to respond have developed clinical diphtheria.

Summary

Active immunization produced a very decided increase of antitoxin in a relatively short time in all persons who had natural antitoxin. These, however, were immune to diphtheria before the injections were made.

In a series of 700 scarlet fever patients of varying ages tested by us for natural immunity by the Schick reaction, 400 gave a negative reaction.

Fifty-seven per cent., therefore, needed neither active nor passive immunization. Less than one-quarter of the remaining 43 per cent., which were probably susceptible to diphtheria, reacted to active immunization with mixtures of diphtheria toxin and antitoxin to a degree sufficient to immunize them surely. A larger percentage developed a trace of antitoxin which was possibly enough to give a slight protection.

CONCLUSIONS

1. Those who are definitely exposed to infection should be passively immunized, even if the toxin-antitoxin injections have been given.

2. The use of the Schick test will eliminate the necessity of immunizing about two-thirds of those subjected to exposure, as, judging from 2 years' experience, those not reacting are immune.

3. Those found to be naturally immune probably continue immune for a considerable period of time, possibly indefinitely.

4. Active immunization is indicated when there is no immediate danger of infection and when it is desirable to lessen the number of susceptible persons.

LATE RESULTS IN THE ACTIVE IMMUNIZATION WITH DIPHTHERIA TOXIN-ANTITOXIN AND WITH TOXIN-ANTITOXIN COMBINED WITH DIPHTHERIA BACILLI

William H. Park and Abraham Zingher

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PART II

The first part of this paper shows the early results which we obtained in the active immunization with mixtures of toxin and antitoxin. This work was carried on in the scarlet fever wards of the Willard Parker Hospital.

In the early stages of the work we soon noted that all individuals, who had a natural antitoxic immunity, produced a very decided increase in the amount of antitoxin after injections of toxin-antitoxin. The response was very prompt and followed even after a single injection. These individuals were, however, naturally immune, and needed no further immunizing injections.

Entirely different results were obtained in the patients who had no natural antitoxic immunity. An early response (within 4 weeks after the injections) to the active immunization was noted in only 25 to 30 per cent. of the injected individuals. The examination of the blood serum of these patients for antitoxin showed the presence of from 1/10 to 10 units per cubic centimeter. Among those who did not definitely respond, however, a number gave a fainter Schick reaction than the control test made before the injections of toxin-antitoxin. This showed that at least a trace of antitoxin had developed in the cells and had accumulated in the blood, which was not sufficient, however, to suppress the Schick reaction. When we retested these individuals from 2 to 18 months later, a large proportion gave now a negative Schick reaction and was thus found to have finally become immune.

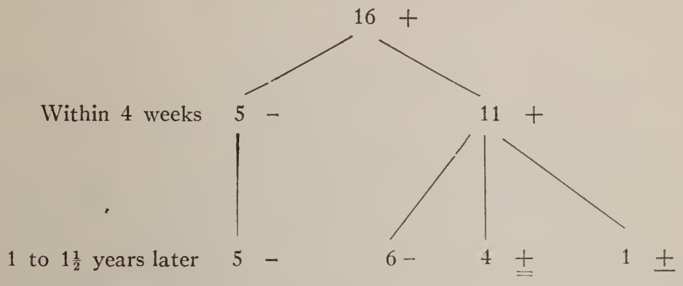
This late development of antitoxin is interesting in connection with observations which we had made with the Schick test in patients who had had diphtheria some weeks previously. Among these we noted that fully two-thirds still gave a positive reaction, thus indicating in these a failure to develop an antitoxic immunity within the period noted after one attack of the disease. These figures corresponded closely with the ones showing the early results of active immunization with toxin-antitoxin. An interesting observation, however, was made in this connection when our chronic tube cases were tested, who had had diphtheria 8 months or more previously. Nearly all gave a negative Schick reaction. This may have been due to a late development of antitoxin or to the fact that the cells only slowly yielded up to the blood the antitoxin formed in small amount soon after the infection. A few of these patients may have been subject to recurrent infections with the diphtheria bacillus. There was, however, no proof of this. In a group of 4 cases, for instance, who had shown a positive Schick reaction after an attack of laryngeal diphtheria, the gradual development of a permanent antitoxic immunity could finally be traced during the succeeding 12 months. In another patient, who had developed an immunity soon after the disease, a persistent negative Schick reaction was obtained during the entire period of observation.

These suggestive findings led to the conclusion that, while the immediate results of attempts at active immunization with toxin-antitoxin were disappointing, later ones, obtained by retesting the immunized individuals without further injections, might give a much better showing. We therefore determined to follow up and retest from 4 to 18 months after discharge from the hospital as many of the injected children as possible. This tardy development of immunity in man is not unexpected, for it is a well-known fact that immunization of certain animals, like guinea pigs, with diphtheria toxin alone or with the toxin-antitoxin mixture is a slow process. It almost seems that in some persons there is not only the absence of antitoxin, but some substance which destroys the first traces of antitoxin entering the lymph and blood, so that some time elapses before an excess of antitoxin becomes apparent in the circulating blood. The considerable success already noted of the tests of persons some months after the toxin-antitoxin injections, led us to continue the work, and also to vary somewhat the methods employed, with the hope of getting even better final results. As is well known, diphtheria has shown during the past decade practically no decrease in morbidity; the mortality of the disease, however, has been greatly lowered by the early administration of antitoxin. The problem, therefore, of an efficient method of active immunization in schools, asylums, hospitals and in the homes remains of great importance. By means of the Schick test we can detect the susceptible individuals and control in a simple and yet accurate way the results of the active immunization.

The individuals who were retested with the Schick reaction to determine a late development of an antitoxic immunity can be divided into three groups:

The early results in Groups I and II will be found in the first part of the paper. From Group II 16 cases were retested from 1 to 1½ years after the injection of toxin-antitoxin.

Early and Late Schick Tests in Group II



— Indicates negative Schick reaction.
$\underset{=}{+}$ " faint " "
± " moderate " "
+ " fully developed Schick reaction.

Five of the 16 who had shown an early and decided production of antitoxin again gave a negative Schick reaction. Of the 11 who had failed to respond at first to the immunizing injections, 6 gave now a negative, 4 a very faint and only 1 a positive Schick reaction.

Group III consisted of 158 patients, who had originally given a positive Schick reaction. These patients were immunized with a mixture of toxin-antitoxin and a vaccine made of killed (57° C. for 45 minutes) diphtheria bacilli. Three to four subcutaneous injections were given at intervals of 3 to 4 days, each injection consisting of 1 c. c. toxin-antitoxin (85 per cent. L + dose of toxin to each unit of antitoxin) and 1,000,000,000 bacteria. One c. c. of the mixture injected subcutaneously into a guinea pig produced a slight local induration and paralysis in 10 days.

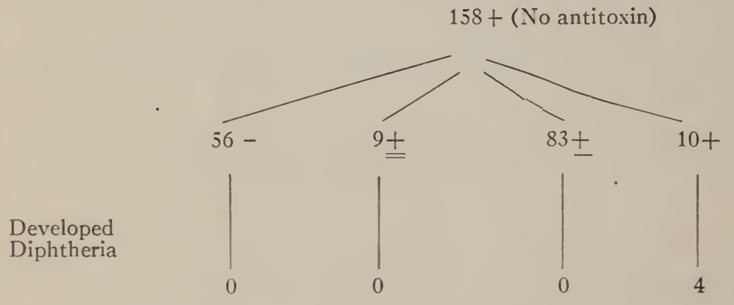
Local and general reactions were noted in about one-half of the injected individuals. They were, as a rule, more frequent and more marked in the older children and in adults than in the young children. These reactions are due in part to the slight excess of free toxin, and in part to the protein substance of the diphtheria bacilli; the more toxic mixtures will give, however, more severe reactions.

Local reactions consisted of pain, redness, some induration and local tenderness at the site of injection. The lymph glands in the axilla were not involved. General reactions consisted of a temperature between 100 and 102 degrees F. and slight malaise. The local signs appeared within 24 hours, lasted for another 24 hours and then gradually disappeared. The temperature continued, as a rule, only for 1 day; in some for 2 days. In no case have any serious effects appeared.

EARLY RESULTS IN GROUP III

158 Cases.

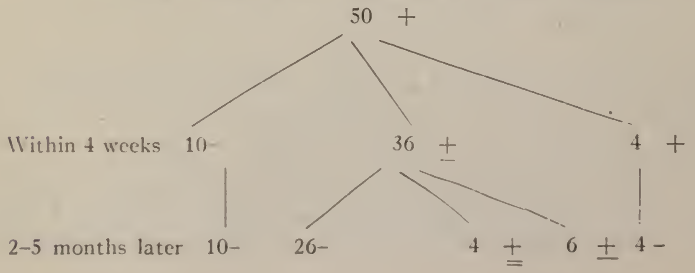
No antitoxin (+ Schick) before immunization.



Of the 158 injected patients, 56, or 35.5 per cent., produced enough antitoxin within four weeks to give a negative, and 9, or 6 per cent., a very faint Schick reaction. Of those who were partly or totally refractory, 83, or 52 per cent., gave a moderately positive, and 10, or 6.5 per cent., a strongly positive Schick reaction. Of the 10, 4 developed clinical diphtheria.

To note any late development of immunity we retested, from 2 to 5 months after the injections, 50 cases of this group:

EARLY AND LATE SCHICK TESTS IN GROUP III

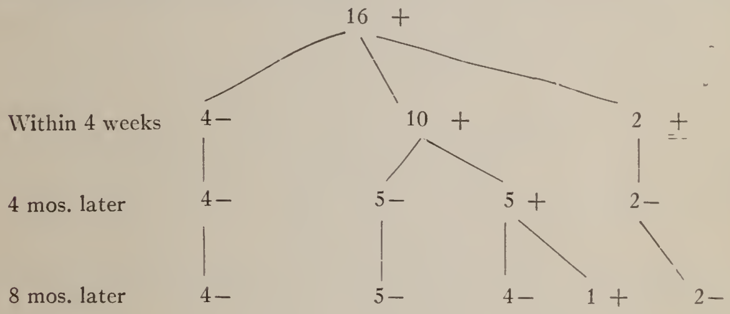


Ten of the patients who had given a definite early production of antitoxin continued to show a negative Schick reaction. Of the 36 patients who had produced only a trace of antitoxin, sufficient only to slightly inhibit the Schick reaction, 26 now gave a negative, 4 a very faint and 6 a moderately positive reaction. The remaining 4, who showed no antitoxin production at first, now gave a negative Schick reaction.

Grouping together the early and late successful results in this series of cases, we find that 40 out of 50 susceptible individuals had developed an antitoxic immunity after the injections of toxin-antitoxin plus vaccine. The remaining 10 gave a reaction which was moderately positive in 6 and faintly positive in 4 cases. These cases probably had also developed some bactericidal immunity from the bacilli injected.

In addition to these, we also immunized with toxin-antitoxin a series of 16 normal children (Group IV), who had given a positive Schick reaction. These children were injected about a year ago, and retested with the Shick reaction 4 and 8 months after the immunization.

EARLY AND LATE SCHICK TESTS IN GROUP IV (NORMAL CHILDREN)

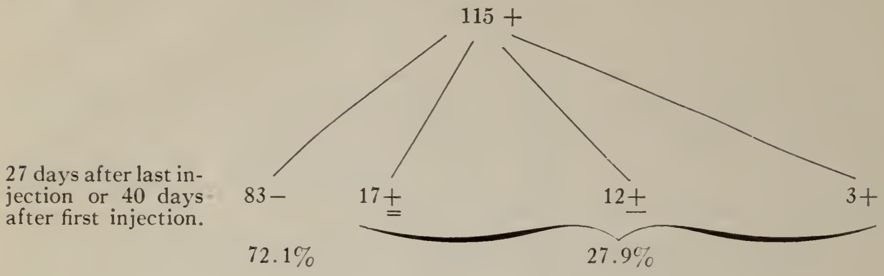


Within four weeks after the injections only 4, or 25 per cent., gave a negative Schick test. Four and eight months later, these 4 still gave a negative reaction, thus indicating a probable lasting immunity. Ten children, who produced no antitoxin at first, now showed the following interesting results: At the end of four months, 5 gave a negative and 5 a positive Schick reaction. At the end of 8 months, 9 showed a negative Schick reaction and only one was still positive. Two of the 16 children who had given a partial early response were found immune at the end of 4 and 8 months.

Sixteen months later, 8 of the actively immunized children were retested; 7 of those who had become immune at the end of 8 months were found to be still protected, while the one who proved resistant again gave a positive Schick reaction.

Finally in one institution (Group V) we immunized, with a slightly toxic mixture of toxin-antitoxin, 115 normal children, from 4 to 16 years of age, who gave a positive Schick reaction. Of these children, 90 received three injections and 25 received two injections of toxin-antitoxin. The dose was 1 c. c. injected subcutaneously and repeated at intervals of 7 days. When the first retest was made, 26 days after the last injection, or 40 days after the first injection, we obtained the following very interesting and encouraging results:

RESULTS IN GROUP V


115 +
27 days after last injection or 40 days after first injection.
83−
17$\underline{\pm}$
12±
3+
72.1%
27.9%

This group shows that fully 72.1 per cent. of children, who had given a positive Schick reaction before the injections, had become actively immunized within a relatively short time after the first injection of toxin-antitoxin.

In one of the institutions we also retested, after 16 months, a number of normal children, who had not been injected with toxin-antitoxin. The following table gives the results of all the retests:

	Not Immunized		Immunized
June, 1914	14−	18+	8+ (toxin-antitoxin)
October, 1915	12−	3−	7−
	1+	15+	1+
	1±		

The table shows that of 14 negatively reacting children between the ages of 5 and 15 years, when retested after 16 months, 2 gave a positive, but 12 still showed a negative reaction. Of 18 originally positive Schick cases, 3 were now negative, while 15 were still positive. It is interesting to compare with these 18 the group of 8 children who had been actively immunized with toxin-antitoxin; their retests showed that a much larger proportion had become immune than could be accounted by the time factor alone.

From the retests in the naturally immune children in this series and in a larger one at present under observation by us, the rather important fact may be deduced that a great majority of children of school age, who show a negative Schick reaction, probably remain protected for years, and even for life. Such persistent immunity, as shown with the test, could be successfully utilized in school children. During an outbreak of diphtheria among children previously tested, it would be necessary to immunize with antitoxin only the susceptible children. Class work could then continue without interruption.

Our results in active immunization up to the present time have led us to the conclusion that, while the toxin-antitoxin injections cannot be depended on to prevent diphtheria in the presence of immediate danger of infection, because of the slow development of immunity in a majority of the cases, yet the injection is of value in rendering a community or school population immune to the danger of infection from future exposure up to a period of possibly several years.

The Research Laboratory will supply those connected with institutions, who are interested in the subject of active immunization against diphtheria, and are willing to furnish us a report of the cases, diphtheria toxin for the Schick test and a mixture of diphtheria toxin-antitoxin for immunization.

A—The reports should include:

(1) The character of the Schick reaction before the injections and at the following intervals after the last injection: 3 weeks, 6 weeks, 3 months, 6 months, 1 year and 2 years. Pseudo-reactions to be carefully controlled with toxin heated to 75° C. for 5 minutes.

(2) The number of injections made: The dose recommended is 1.0 c. c., injected three times at intervals of 7 days. The injections are made subcutaneously in the arm over the insertion of the deltoid. The dose is the same for individuals from one year to adult life. Infants below the age of 12 months receive 0.5 c. c. with each injection.

(3) The character of the local and general reactions, if any, after each injection.

(4) A description of the clinical cases of diphtheria that develop among the injected individuals during the course of immunization or afterward. Culture reports for the Klebs-Loeffler bacillus, etc.

(5) Where possible, a quantitative estimation of the amount of antitoxin developed as a result of immunization.

B—Groups of individuals recommended for active immunization:

Only positive Schick cases to be injected:

(*a*) Children from 1 to 6 years of age.

(*b*) Children in schools, orphan asylums and various other institutions.

(*c*) Mothers and infants in lying-in places.

(*d*) Physicians, nurses and ward workers in contagious disease hospitals.

(*e*) Patients and nurses in general hospitals.

Active Immunization by Means of Killed Cultures of Diphtheria Bacilli

Following the work with mixtures of diphtheria toxin-antitoxin, and before beginning the combined immunization with toxin-antitoxin and diphtheria bacilli, we injected a series of children only with vaccines made from killed diphtheria bacilli.

We immunized 210 scarlet fever patients, who gave a positive Schick test and were exposed to diphtheria, with vaccines of the Klebs-Loeffler bacillus (heated to 57° C. for 45 minutes). Three to five injections of 2,000,000,000 bacteria each were given at intervals of 4 to 5 days. Local reactions were generally noted, occasionally associated with a slight rise in temperature.

Of the 210 patients that were thus treated, 18 deveolped clinical diphtheria (Tonsillar, 8; Nasal, 9; Laryngeal, 1).

After 1 vaccine injection in 14 cases immunized 4 developed diphtheria.
After 2 vaccine injections in 15 cases immunized 4 developed diphtheria.
After 3 vaccine injections in 19 cases immunized 4 developed diphtheria.
After 4 vaccine injections in 28 cases immunized 3 developed diphtheria.
After 5 vaccine injections in 134 cases immunized 3 developed diphtheria.

Thirty of the remaining patients showed positive throat cultures with virulent bacilli, but had no clinical symptoms.

These results with the vaccine are rather discouraging, especially in view of the claims made by several authors of the therapeutic value of such vaccines in carriers. Not only did we fail with these vaccines in ridding carriers of their organisms, but we even failed to immunize about one-quarter of the injected individuals against becoming carriers or developing clinical symptoms.

Conclusions

1. Individuals who, before treatment, gave a negative Schick reaction are immune, probably for life, and, therefore, it is not necessary to inject them when exposed either with antitoxin or toxin-antitoxin.

2. Those who give a positive Schick reaction and are exposed to diphtheria and in immediate danger, should receive either antitoxin alone or, if a longer protection is desired, both antitoxin and toxin-antitoxin.

3. For the general prophylaxis against diphtheria in schools and communities, excluding immediate contacts, a mixture of toxin-antitoxin alone (85 per cent. to 90 per cent. of the L+ dose of toxin to each unit of antitoxin) or toxin-antitoxin plus vaccine of killed diphtheria bacilli is recommended. The dose is 1 c. c. of toxin-antitoxin and 1,000,000,000 bacteria injected intracutaneously and repeated three times at intervals of 6 to 7 days. Sufficient time has not as yet elapsed to judge the value of adding the injections of the bacilli to the toxin-antitoxin.

4. The early and the late results of active immunization should be determined with the Schick test. Early results are those obtained by the application of the test within 4 weeks, late results from 2 months to 2 years after the immunizing injections.

NITRIC ACID COMPARED WITH TINCTURE OF IODIN IN THE CAUTERIZATION OF WOUNDS INFECTED WITH RABIES VIRUS

DANIEL W. POOR

In a previous report it has been shown that rabies could be prevented in 41 2/3 per cent. of guinea pigs in which the wounds were subsequently cauterized with fuming nitric acid 22 hours after infection with the virus. In that experiment the infecting agent was an emulsion of the brains of dogs which had succumbed to street virus. For the reason that it may be objected that possibly the brain virus does not travel as rapidly from the point of inoculation as the salivary virus, it was determined to vary the experiment by using the latter virus thus making the test conform as nearly as possible to clinical conditions. Twenty-four (24) guinea pigs were used as test animals.

Technique—The hair of the back of the neck was removed by barium sulphide and an incision 1/2 to 2/3 inch long made with a scalpel, the subcutaneous tissues being cut on either side in several places with scissors, thus making a lacerated wound involving the nerve structures similar to that made by a dog's teeth. The virus, which was obtained from the salivary glands of three rabid dogs according to the aspiration method described in *The Journal of Infectious Diseases,* Vol. XIII, No. 2, was rubbed into the wounds on cotton swabs, a separate swab being used for each animal. The wounds were then brought together with a ligature.

At the end of 24 hours the ligatures were removed in all except eight pigs which were used as controls. After etherization, fuming nitric acid was applied thoroughly to the wounds of eight of the pigs, on cotton swabs similar to those used in infecting the animals. The excess of the acid was removed with a dry swab. In eight pigs the wounds were treated with tincture of iodin in the same manner. In four the excess of iodin was removed and in four it was allowed to remain.

The result of the experiment was as follows:

Nitric Acid	Tinct. of Iodin	Controls
1. Discharged after 6 weeks.......	Rabies in 12 days.........	Rabies in 13 days
2. Discharged after 6 weeks.......	Rabies in 16 days.........	Rabies in 14 days
3. Discharged after 6 weeks.......	Rabies in 29 days.........	Rabies in 20 days
4. Discharged after 6 weeks.......	Rabies in 18 days.........	Rabies in 14 days
5. Discharged after 6 weeks.......	Rabies in 14 days.........	Rabies in 13 days
6. Rabies in 22 days..............	Rabies in 19 days.........	Rabies in 14 days
7. Rabies in 23 days..............	Rabies in 13 days.........	Discharged after 6 weeks
8. Rabies in 37 days..............	Rabies in 19 days.........	Discharged after 6 weeks

75 per cent. of the controls died after an average incubation of 14 2/3 days. 37½ per cent. of the animals cauterized with nitric acid died, indicating a saving of 37½ per cent. by means of the acid. It is to be noted that two of the pigs in this group which died of rabies showed an incubation of 22½ days as compared with 14 2/3 days for the controls and that the other had the extremely long incubation of 37 days—a lapse of time that would permit of a course of Pasteur treatment with a subsequent interval of

two weeks for the full development of immunity. The pigs whose wounds were treated with tincture of iodin showed a mortality of 100 per cent.—25 per cent. more than the controls. Seven of the animals had an incubation of 18 5/7 days and one an incubation of 29 days.

One cannot be sure from the results of this experiment that the application of tincture of iodin as late as 24 hours after infection actually does harm. Too few animals have been used and the difference in mortality is too slight to prove this, especially, in view of the fact that rabies infection is always more or less a matter of uncertainty. It is evident, however, that the application of the iodin so long after infection did no good and it is possible that an agent such as this may hinder the tissue cells in their struggle against the virus and at the same time lack the ability to destroy all the rabies organisms after these have penetrated into the tissues. It cannot further be assumed that tincture of iodin may not be of use when thoroughly applied soon after the bite, before the virus has reached a point in the nerve tissue where it cannot be destroyed by the iodin. On the other hand, it is evident that the nitric acid is of considerable use when thoroughly applied after a lapse of twenty-four hours. The percentage of animals saved is about the same as in the experiment previously reported (37.5 per cent. as against 41 2/3 per cent.) The good effect of the acid is undoubtedly due to its penetrating destructive action which enables it to reach every portion of a ragged wound and exert its action somewhat beyond the actual point of contact.

It is quite possible that the efficiency of nitric acid may not be as great in wounds of human beings as in those of small guinea pigs, as the tissues of the latter are probably more thoroughly destroyed by the acid. Cabot showed, however, that the efficiency of nitric acid in guinea pigs was much greater than that of the actual cautery or silver nitrate. Its superiority over tincture of iodin is shown in the above experiment. In man nitric acid would be relatively superior to these other agents, if not absolutely as efficient as in guinea pigs. Nitric acid has been used freely in the clinic at the Health Department for several years. No difficulty is experienced in the subsequent healing of the wounds if one avoids applying the acid to the periosteum. Healing may be considerably delayed in the case of wounds on the legs in elderly persons in whom the circulation is poor, but in this situation it is seldom necessary to use such a severe cauterization. It has been our custom to use the acid in wounds considered dangerous on account of extent or location, especially those on the head or hands, employing local or light general anaesthesia when this is advisable. Chloroform anaesthesia is often to be recommended in the case of children.

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EXPERIMENTS ON THE COMPARATIVE ANTIRABIC IMMUNITY PRODUCED BY THE PASTEUR VIRUS, THE HARRIS DESICCATED VIRUS AND THE CUMMING DIALYZED VIRUS

Daniel W. Poor, Assisted by Frederick Jelinek and Carl Gilson

The immunity afforded by the Pasteur antirabic virus was compared with that produced by two methods introduced in recent years, viz., that devised by Harris of St. Louis, and that by Cumming of Ann Arbor. The tests were in the main carried out so as to give an idea of the relative speed with which it was possible to develop immunity by the three methods; in two experiments, however, the test was for *duration* of immunity. Rabbits were used and these were treated, in most of the experiments, for 9 or 10 days, being then injected intracerebrally with ¼ c. c. of different dilutions of fixed virus on the day of the last injection or on the succeeding day. Protocols of ten characteristic experiments follow:

Experiment I

Pasteur and Harris Methods

Animals in each series treated 10 days.

Harris Virus: Sent from Dr. Harris' Laboratory; age of virus about 2 years. M.I.D. supposed to be 0.1 mg. for the first day's treatment and 0.01 mg. for that of succeeding days, but according to our tests they were much lower. Exact M.I.D. not determined by us. The animals treated with this virus received the following doses which constitute the ordinary scheme proposed by Dr. Harris for treatment of human beings:

1st day— 500 units.
2d day—1,000 units.
3d day—1,500 units.
4th day—2,000 units.
5th day—3,000 units.
6th day—3,000 units.
7th day—3,000 units.
8th day—3,000 units.
9th day—3,000 units.
10th day—3,000 units.

Unless otherwise stated, this scheme was followed in all subsequent experiments.

The rabbits in the Pasteur series received the full doses given to human patients on the first ten days of the regular course of Pasteur treatment.

Forty-three days after the end of treatment, animals were infected intracerebrally with the following dilutions of fixed virus.

1—8,000—
Harris Virus:
Rabbit No. 1—Discharged after 7 weeks.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 13 days.
Rabbit No. 2—Paralyzed in 7 days.
Controls:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.

1—4,000—
Harris Virus:
Rabbit No. 1—Remained well for 32 days, then developed snuffles and died.
Rabbit No. 2—Discharged after 7 weeks.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 6 days.
Control:
Rabbit No. 1—Paralyzed in 7 days.

1—1,000—
Rabbits of all series showed beginning symptoms of rabies in 6 days.

EXPERIMENT II

Pasteur and Harris Methods

Animals treated 11 days.

Harris Virus sent from Dr. Harris' Laboratory. M.I.D. for first day's treatment 0.1 mg.; that of virus for succeeding days 1/80 mg. Animals infected the day after the last injection with the following dilutions of fixed virus:

1—12,000—
Control:
Rabbit No. 1—Paralyzed in 10 days.
Rabbit No. 2—Paralyzed in 7 days.
Pasteur Virus:
Rabbit No. 1—Discharged 40 days later.
Rabbit No. 2—Dead in 7 days—probably of snuffles.
Harris Virus:
Rabbit No. 1—Discharged in 40 days.
Rabbit No. 2—Discharged in 40 days.

1—8,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 8 days.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 8 days.
Rabbit No. 2—Paralyzed in 8 days.
Harris Virus:
Rabbit No. 1—Discharged after 40 days.
Rabbit No. 2—Discharged after 40 days.

1—4,000—
Control:
Rabbit No. 1—Paralyzed in 8 days.

Pasteur Virus:
Rabbit No. 1—Paralyzed in 8 days.

Harris Virus:
Rabbit No. 1—Discharged after 40 days.

EXPERIMENT III

Harris Series—Treated 10 days.

Pasteur Series—Treated 21 days. Starting 11 days ahead of the Harris Series.

Harris Virus: Virus used for the first day's treatment made by Eli Lilly Company, M.I.D. .1 mg.; that for succeeding days made by us. M.I.D. 1/200 mg. Virus 1 month old. On the day after the last injection all animals infected with the following dilutions of fixed virus:

1—8,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Harris Virus:
Rabbit No. 1—Discharged after 38 days.
Rabbit No. 2—Paralyzed in 17 days.
Pasteur Virus:
Rabbit No. 1—Discharged after 38 days.
Rabbit No. 2—Paralyzed in 10 days.

1—4,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Harris Virus:
Rabbit No. 1—Discharged after 38 days.
Rabbit No. 2—Paralyzed in 7 days.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 6 days.
Rabbit No. 2—Paralyzed in 7 days.

EXPERIMENT IV

Pasteur, Harris and Cumming Virus

Harris Virus for the first day's treatment obtained from Eli Lilly Company; M.I.D. 1/10 mg. That for subsequent doses made at Health Department: M.I.D. 1/240 mg.; age of virus 47 days.

The Cumming Virus was made in 1—100 dilution, and injected in dose of 2½ c. c. each day.

All animals were treated 11 days and infected on the day after the last treatment with the following dilutions of fixed virus:

1—12,000—
Control:
Rabbit No. 1—Discharged at the end of 13 days.
Rabbit No. 2—Paralyzed in 6 days.
Cumming Virus:
Rabbit No. 1—Paralyzed in 6 days.
Rabbit No. 2—Paralyzed in 7 days.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 8 days.
Rabbit No. 2—Discharged at the end of 13 days.
Harris Virus:
Rabbit No. 1—Paralyzed in 9 days.

1—8,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Cumming Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Pasteur Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Harris Virus:
Rabbit No. 1—Discharged after 13 days.
Rabbit No. 2—Discharged after 13 days.

The experiment was terminated 13 days after infection owing to the fact that one of the rabbits had developed a large abscess on the neck, necessitating the destruction of the animal.

EXPERIMENT V

Pasteur and Harris Series

Pasteur animals treated 21 days.
Harris animals treated 10 days.

Harris Virus: That for first day's treatment received from Eli Lilly Company; M.I.D. 0.1 mg.; that for succeeding days made at Health Department. M.I.D. 1/240 mg.; age of virus 97 days. Sixty-one (61) days after last injection all animals were infected intracerebrally with the following dilution of fixed virus:

1—7,000—
Pasteur Virus:
Rabbit No. 1—Paralyzed in 9 days.
Rabbit No. 2—Paralyzed in 9 days.
Rabbit No. 3—Paralyzed in 9 days.
Rabbit No. 4—Paralyzed in 9 days.
Rabbit No. 5—Paralyzed in 9 days.

Four (4) rabbits died of snuffles and diarrhoea before infection.

Harris Virus:
Rabbit No. 1—Paralyzed in 9 days.
Rabbit No. 2—Paralyzed in 9 days.
Rabbit No. 3—Paralyzed in 9 days.
Rabbit No. 4—Paralyzed in 16 days.
Rabbit No. 5—Paralyzed in 9 days.
Rabbit No. 6—Paralyzed in 9 days.
Rabbit No. 7—Discharged 58 days later.

Two (2) rabbits died during treatment.

Control:
Rabbit No. 1—Symptoms in 7 days.

Immunity sufficient to protect against subdural injection of the amount of virus used was evidently not present in either series, 61 days after the end of treatment.

EXPERIMENT VI

Harris Virus

Made at Health Department. Age of virus 5½ months. M.I.D. 1/240 mg. Animals treated 11 days and tested intracerebrally on the day of the last treatment with ¼ c. c. of the following dilution of fixed virus:

1—7,000—
Rabbit No. 1—Dead in 7 days.
Rabbit No. 2—Paralyzed in 14 days.
Rabbit No. 3—Paralyzed in 9 days.
Rabbit No. 4—Paralyzed in 9 days.
Rabbit No. 5—Paralyzed in 9 days.
Rabbit No. 6—Dead 58 days later—not rabies.
Control:
Rabbit No. 1—Paralyzed in 8 days.

Record of Treatment—
1st day— 500 units.
2d day—1,000 units.
3d day—1,500 units.
4th day—2,000 units.
5th day—3,000 units.
6th day—3,000 units.
7th day—3,000 units.
8th day—3,000 units.
9th day—3,000 units.
10th day—3,000 units.
11th day—3,000 units.

It would seem that the bad results in this experiment were probably due to the virus not being completely dried and to the subsequent loss in immunizing power resulting from its being kept 5½ months before being used.

Experiment VII

Harris Virus and Cumming Virus 1—50 *Dilution*

From a verbal communication from Dr. Cumming, it was learned that the technique of the manufacture of the dialysed virus had been changed somewhat, the most important change being the increase in the strength of the emulsions from 1 per cent. to 2 per cent.

In this experiment treatment was carried on for 9 days.

Harris Virus: That for the first day's treatment was received from the Eli Lilly Company; M.I.D. 0.1 mg.; the virus for the treatment on subsequent days was made at the Health Department; M.I.D. 0.01 mg.; age of virus 25 days. The animals were tested on the day following the last injection with the following dilutions of fixed virus:

1—12,000—
Control:
Rabbit No. 1—Paralyzed in 9 days.
Rabbit No. 2—Paralyzed in 8 days.
Rabbit No. 3—Discharged after 38 days.
Harris Virus:
Rabbit No. 1—Discharged after 38 days.
Rabbit No. 2—Discharged after 38 days.
Rabbit No. 3—Discharged after 38 days.
Cumming Virus:
Rabbit No. 1—Paralyzed after 13 days.
Rabbit No. 2—Discharged after 38 days.
Rabbit No. 3—Discharged after 38 days.

1—8,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Rabbit No. 3—Paralyzed in 9 days.
Harris Virus:
Rabbit No. 1—Paralyzed in 9 days.
Rabbit No. 2—Paralyzed in 9 days.
Rabbit No. 3—Discharged after 38 days.
Cumming Virus:
Rabbit No. 1—Discharged after 38 days.
Rabbit No. 2—Discharged after 38 days.
Rabbit No. 3—Discharged after 38 days.

Experiment VIII

Harris Virus, Cumming Virus 1—100 *Dilution; Cumming Virus* 1—50 *Dilution*

Harris Virus made at Health Department; M.I.D. 1/200 mg.; virus 6 weeks old. Record of treatment as follows:

Harris Virus:	*Cumming Virus:*
1— 500 units.	2½ c.c.
2—1,000 units.	2½ c.c.
3—1,500 units.	2½ c.c.
4—2,000 units.	2½ c.c.
5—2,500 units.	3 c.c.
6—3,000 units.	2½ c.c.
7—3,000 units.	2½ c.c.
8—3,000 units.	3 c.c.
9—2,500 units.	2½ c.c.

The Harris and Cumming viruses were made in double quantity—every other day and stored with .2% carbolic added.

Infection on the day after the last injection with the following dilution of fixed virus:

1—12,000—
Cumming Virus—1—100:
Rabbit No. 1—Paralyzed in 6 days.
Rabbit No. 2—Paralyzed in 7 days.
Harris Virus:
Rabbit No. 1—Discharged 4 weeks later.
Rabbit No. 2—Paralyzed in 8 days.

Cumming Virus—1—50:
Rabbit No. 1—Paralyzed in 8 days.
Rabbit No. 2—Discharged 4 weeks later.
Control:
Rabbit No. 1—Not completely injected—paralyzed in 24 days.
Rabbit No. 2—Paralyzed in 6 days.

1—8,000—
Cumming Virus—1—100:
Rabbit No. 1—Paralyzed in 8 days.
Rabbit No. 2—Paralyzed in 7 days.
Harris Virus:
Rabbit No. 1—Discharged 4 weeks later.
Rabbit No. 2—Discharged 4 weeks later.
Rabbit No. 3—Discharged 4 weeks later.
Cumming Virus—1—50:
Rabbit No. 1—Paralyzed in 9 days.
Rabbit No. 2—Discharged 4 weeks later.
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Rabbit No. 3—Paralyzed in 9 days.
Rabbit No. 4—Paralyzed in 9 days.

Experiment IX

Cumming Virus 1—50 *and Harris Virus*

Both viruses made up every other day and stored with .2 per cent. carbolic. Harris virus made at Health Department; M.I.D. 1/200 mg.; virus 39 days old. Treatment 9 days, as follows:

Cumming Virus:	*Harris Virus:*
Oct. 20th—2.5 c.c.	500 units—M.I.D. 1/200.
Oct. 21st—2.5 c.c.	1,000 units.
Oct. 22d—2.5 c.c.	1,500 units.
Oct. 23d—2.5 c.c.	2,000 units.
Oct. 24th—2.5 c.c.	3,000 units.
Oct. 25th—3 c.c.	3,000 units.
Oct. 26th—3 c.c.	3,000 units.
Oct. 27th—2.5 c.c.	3,000 units.
Oct. 28th—2.5 c.c.	3,000 units.

Animals tested for immunity on day after last injection with following dilutions of fixed virus:

1—11,000—
Control:
Rabbit No. 1—Paralyzed in 8 days.
Rabbit No. 2—Paralyzed after 7 days.
Rabbit No. 3—Paralyzed after 7 days.

Harris Virus:
Rabbit No. 1—Paralyzed in 6 days.
Cumming Virus:
Rabbit No. 1—Discharged 6 weeks later.
Rabbit No. 2—Discharged 6 weeks later.
Rabbit No. 3—Ataxia 13 days—Paralysis 14 days.

1—7,000—
Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Ataxia in 7 days.
Rabbit No. 3—Ataxia in 6 days.
Cumming Virus:
Rabbit No. 1—Discharged 6 weeks later.
Rabbit No. 2—Paralyzed 9 days later.
Rabbit No. 3—Ataxia in 7 days.

Harris Virus:
Rabbit No. 1—Discharged 6 weeks later.
Rabbit No. 2—Discharged 6 weeks later.
Rabbit No. 3—This rabbit was paralyzed in the hind legs immediately after inoculation. The paralysis continued until time of animal's death, 17 days later; two (2) guinea pigs inoculated subdurally from this rabbit's brain showed no rabies.

Experiment X

Cumming Virus (kept in ice box 25 days) *and Pasteur Virus*

The Cumming Virus was from the lot same as that used in the last experiment. All animals treated 9 days. Injected the day after the last infection with the following dilutions of fixed virus:

1—11,000—

Control:
Rabbit No. 1—Paralyzed in 7 days.

Pasteur Virus:
Rabbit No. 1—Paralyzed in 10 days.
Rabbit No. 2—Paralyzed in 7 days.

Cumming Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.

1—7,000—

Control:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Rabbit No. 3—Paralyzed in 6 days.

Pasteur Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Rabbit No. 3—Paralyzed in 7 days.

Cumming Virus:
Rabbit No. 1—Paralyzed in 7 days.
Rabbit No. 2—Paralyzed in 7 days.
Rabbit No. 3—Ataxia in 18 days.

It is evident from this experiment that keeping the Cumming Virus 3½ weeks in the ice box has materially reduced its immunizing properties.

Compilation of the Foregoing Experiments

	Dead	Lived	Per Cent. Lived
Infection Late			
Harris Virus	6	4	40
Pasteur Virus	9	..	..
Controls	4	..	..
Infection Early			
Harris Virus	12	20	62
Pasteur Virus	9	3	12
Cumming 1-100	8	..	..
Cumming 1- 50	6	10	62
Control	32	2	5

In three experiments in which the Harris Virus and the 2 per cent. Cumming Virus were compared directly the total result was as follows:

	Died	Recovered	Per Cent. Lived
Harris	4	10	71
Cumming	6	10	62

The total recovery percentage of the Harris Virus for all experiments was lowered by the result of one experiment (No. 6), in which the virus used was five and one-half (5½) months old. It is possible that deterioration may have occurred in this virus or that it was not completely dried. It is to be noted therefore that the immunity of 9 or 10 days' treatment by these two methods followed by immediate infection is in general practically the same and that it is superior to that obtained by the Pasteur method. It should be further noted that the full course of treatment by the Pasteur and Cumming methods lasts 21 days. In these experiments the treatment was limited to 10 days and the test of immunity made very severe (intracerebral) in order to test the three methods on the most serious type of case with which we have to deal, viz., head infections with short incubation.

TECHNIQUE OF PRODUCTION OF VIRUS

Harris Method

The technique employed was that advocated by Dr. Harris with a few minor variations. One modification used by us may be mentioned. Immediately after the frozen powder has been placed in the dish, which in turn is to be placed in the desiccator, it is covered by a sterile cap secured in place by adhesive plaster. The cap is made of a thin layer of common cotton stitched between two layers of gauze and is later sterilized. The idea of this cap is to prevent as far as possible the entrance of germs when air is let into the desiccator, preparatory to removing the cover. An experiment which indicated the advantage of this procedure is the following: Melted agar was poured into the sterile virus dish, which was then placed in the desiccator uncovered. The requisite vacuum was made and then the air allowed to rush in through the sulphuric acid and calcium chloride apparatus used for drying the air. After incubation of the agar eleven (11) colonies developed. The same process repeated with a dish covered with a cap resulted in no development of bacteria. The cap does not appear to interfere with the drying of the virus provided too much cotton is not used. With proper attention to details the technique of the production of this virus is not difficult. No difficulty should be experienced in securing sufficient snow. The grinding and freezing of the virus should be done in a small room which can be kept clean and free from air currents. The one who does the grinding should wear sterile gloves and gown. Some difficulty may be found in securing the requisite vacuum. A good Geryk pump is necessary with adequate arrangement for drying the air entering the pump. The Scheibler jars must be well ground. The seal we have found most useful is made of pure gum rubber 3 parts, beeswax 1 part.

Cumming Method

The dialyzed virus was made as at present recommended by Dr. Cumming. A 2 per cent. emulsion of fixed virus brain is made in distilled water. After standing one hour the supernatant fluid is decanted off and .1 per cent. formalin added to it. After standing for at least three hours the virus is placed in collodion sacs and the formalin removed by dialysis. With the apparatus used by us this required 15 to 18 hours. After removal of the virus from the sacs .2 per cent carbolic acid was added. In our experiment the virus was made freshly every other day, except in the one instance where it was purposely kept 25 days. We used sacs 18 inches long by 1½ inches in diameter. They may be sterilized in an autoclave or by boiling. In our experiments on rabbits the sacs were for the most part not sterilized. No abscesses resulted. Eight (8) different lots of dialyzed virus were tested for virulence and all found to be non-virulent.

STANDARDIZATION OF HARRIS VIRUS

Since rabies virus is a suspension of solid particles and not a solution, and since its intracerebral injection in the test animals involves a somewhat complicated technique, it follows that the tests for the standardization of its virulence must show more or less irregular results. We have obtained the best results with normal guinea pigs weighing from 250 to 300 gms. An emulsion of the strength of 1 mg. to 1 c. c. is made, the virus being ground as fine as possible. This is the stock emulsion from which dilutions are made. Each animal receives ¼ c. c. intracerebrally. A ¼ c. c. Record syringe with fine needle is convenient for this work. For example, if 1 c. c.

of the stock solution be diluted in 59 c. c. salt solution and ¼ c. c. injected, the animal receives 1/240 mg.

It is to be noted that while the M.I.D. of most of the virus may be considered to be from 1/200 to 1/240 mg. occasionally a lot is made in which the virulence falls far below this. The reason for this is probably that the virus is not completely dried, although it may appear to be so.

Bacteriological Tests

The following are some tests of the sterility of the Harris virus:

1. 10 to 15 mg. of virus planted in each of three broth tubes and 30 to 40 mg. planted on each of two agar plates. The broth tubes remained clear. After 24 hours one colony appeared in the plates. After five days the two plates showed ten colonies. These proved to be cocci, which, when inoculated in pure broth culture in two guinea pigs in the amount of 2½ and 3 c. c., produced no ill effect.

2. About the same amount of virus as above, planted in two broth tubes and three plates. Both tubes showed growth; one plate remained sterile and one contained four colonies of cocci. The broth culture was inoculated in the guinea pigs as follows:

Pig No. 1—1 c. c., discharged 38 days later.
Pig No. 2—2 c. c., dead 37 days later.

3. About 30 mg. were sown on each of three agar plates and in three broth tubes. After five days' incubation one plate was sterile, the other contained two colonies. Two of the broth tubes showed growth, one remained sterile. Two pigs inoculated with ½ and 1 c. c. of the broth culture respectively, were discharged 38 days later. Several tests of samples of snow showed them to be sterile. No cultures were made of the dialyzed virus, as there should be no difficulty in keeping this sterile.

Conclusions

1. Both the desicated and 2 per cent. dialyzed virus (Harris Virus and Cumming Virus) produce immunity more quickly than the Pasteur virus. The 1 per cent. dialyzed virus gave practically no immunity in the tests made by us.

2. The Harris virus possesses the advantage of holding its virulence and immunizing property for several months. The technique of its manufacture is, however, somewhat complicated and slight contamination with air bacteria is apt to occur.

3. The Cumming dialyzed virus possesses the advantage of being non-virulent, but in our experience loses in immunizing power rather quickly when kept more than a few days. The fact that these methods appear to immunize about equally rapidly and successfully coupled with the fact that the one contains ten times as many infective units as the full Pasteur treatment, while the other is non-virulent, suggests the idea that virulence has very little to do with antirabic immunity.

Assuming that practically all the moisture is removed from the Harris virus, an estimate of the actual amount of nerve tissue injected in ten days' treatment varies between 115 and 140 mg.

Based on Halliburton's figures for the proportion of solid matter, for different portions of the nervous system, one may assume that the amount of nerve tissue injected in a course of ten days' treatment by the 2 per cent. dialyzed brain method is represented by 100 mg. It is difficult to compute the amount of solid matter in the Pasteur series, because the portions of cord are neither completely dried nor fresh and the proportion of water to solid matter varies with each day's treatment.

It has been shown that, weight for weight, the fixed virus brain is many times more infectious than the cord. This may be due to a larger number of rabic organisms in the former location. In the Harris virus the relative weight of brain and cord is roughly as 3 to 1. In the Cumming method all the virus is made from brain tissue, while in the Pasteur method only the cord is used. It would seem from the foregoing fair to conclude that the brain tissue is superior to the cord in immunizing properties, irrespective of whether the organisms contained therein are living or dead.

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THE USE OF CONVALESCENT AND NORMAL BLOOD IN THE TREATMENT OF SCARLET FEVER

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Beneficial effects have been obtained at the Willard Parker Hospital during the past seven months in the treatment of severe forms of scarlet fever with intramuscular (1) injections of fresh human blood, either convalescent or normal. In a number of such patients, where the prognosis seemed doubtful and occasionally even absolutely poor, the life saving effect of 6 to 8 ounces of blood, repeated once or twice at intervals of 4 to 5 days, has encouraged us sufficiently to recommend the treatment in the severer forms of the disease.

The intravenous injection of fairly large quantities (2–3½ ounces) of convalescent serum has given good results in the hands of Reiss and Jungman (2), and of R. Koch (3). This requires greater skill, and necessitates wasting more than 60 per cent. of the blood. These disadvantages are easily avoided by the simple intramuscular injection of whole citrated blood, a method described by me in a previous communication, especially as it has been shown by Meltzer and Auer, and by Morgeuroth and Levy, that absorption from the muscle is very much faster than it is from subcutaneous tissue. In fact, the rapidity of action of substances so injected approximates very closely that following an intravenous injection.

Cases of scarlet fever may be divided roughly into two groups for purposes of blood injection:

(1) The early toxic or malignant scarlet fever cases, which are seen between the first and fourth day of disease, and show the clinical picture of delirium and restlessness, intensive purple or petechial rash, moderate glandular enlargement and severe angina, with a slight exudate over the tonsils, uvula, and pillars of the fauces. These cases are suitable for the intramuscular injection of *convalescent* blood, taken from the second or third week convalescent scarlet fever cases. Such convalescents are generally available in the wards of large contagious disease hospitals. They should be free from syphilis and tuberculosis. A record of individuals, who are willing to act as donors for a suitable consideration, can be kept by the hospital authorities. The donors themselves, or the blood taken from them freshly and citrated, may be sent to the homes of private patients, and the treatment can be thus made available for use in general practice. Convalescent blood will deteriorate after a month or two when kept in the ice-box, and it is, therefore, preferable to use only fresh blood. The question of using donors who are several months convalescent may be considered and should be tried on a larger scale.

The effect of convalescent blood in these early toxic or malignant cases of scarlet fever is seen in a critical drop in temperature beginning about six hours after the injection and ending in 24 to 30 hours. The temperature often reaches normal and will remain nearly normal in the majority of the cases; in others, especially when complicated with severe tonsillar and faucial exudate, associated with inflamed cervical glands, the temperature may rise again for a few days, but rarely to the same height as at the time of injection. Other results, which are quite as striking, can be seen in the early fading of the rash, the improvement in the circulation and the character of the pulse, the general condition and mental symptoms. The delirium will frequently disappear, and the clinical eye detect that general improvement, which is so difficult to interpret by a mere temperature, pulse and respiration record.

(2) In the second group are included the later septic cases, seen from the 5th to the 8th day of disease. The rash may have faded entirely, but the membranous exudate over the fauces and tonsils is severe and extensive, and often appears necrotic. The cervical lymph glands are enlarged and tender. The temperature is high and septic in character, varying between 103 to 105 degrees Fahrenheit, the pulse is proportionately small and rapid. A diffuse duskiness is often noticeable over the entire body surface. Pulmonary symptoms may or may not be present. The ears are frequently discharging.

In this group of cases, we would strongly recommend the use of fresh *normal* blood which can be readily obtained from one or both of the parents, or some other close relative of the patient. The intramuscular injection of four ounces of blood in children up to the age of four years, of 6 to 8 ounces of blood in older children and adults will give most satisfactory results. These blood injections may have to be repeated once or twice at intervals of 4 to 5 days. Such repeated injections are made in the same muscles, since the blood is absorbed with great facility. Not much specific effect is claimed for these injections of fresh normal blood; but the nutritive and stimulating properties and normal antibody content of relatively large amounts of normal blood have shown definite beneficial results in some desperately ill cases, and have convinced us that the treatment of septic cases, with a doubtful or poor prognosis, should invariably include the administration of such a harmless and yet frequently efficient remedy.

The method of obtaining the blood, and its injection is very simple; with a one ounce (30 c. c.) Record syringe, a Luer Syringe or, in an emergency, any easy working large syringe, and a medium sized needle (preferably a No. 17 or No. 18 gauge platinum iridium needle) the necessary quantity of blood is rapidly aspirated from the mediancephalic vein of the donor at the bend of the elbow, and immediately citrated by adding the blood to a 10 per cent. solution of sodium citrate in the proportion of one ounce of blood to each c. c. of the citrate solution. This makes the final dilution of the citrate 0.33 per cent. The needle inserted into the vein of the donor should not be removed; the syringe is detached when full, while an assistant carefully fixes the hub of the needle and attaches a 5 c. c. Record Syringe containing some 1 per cent. sodium citrate solution to keep the needle free of blood. Three to four syringefuls of blood may thus be obtained, before it is necessary to rinse out the larger syringe with a 1 per cent. solution of citrate which is kept ready in a beaker. We prefer to collect the blood in 100 c. c. bottles, each of which contains 2 c. c. of the 10 per cent. citrate solution. To each bottle, two ounces of blood is added, shaking the bottle after each addition to distribute the sodium citrate solution. In less than ten minutes, the required amount of blood (from 4 to 10 ounces) is obtained and is then ready for injection into the patient.

The blood is injected into the following regions: triceps, outer regions of both thighs (vastus externus), the calves (soleus) and both gluteal regions. In young children, half an ounce, in older children, and adults, one ounce is injected into each of these muscles.

The blood serum is rapidly absorbed, as shown by the soft and supple condition of the muscles on the following day, when they will be found to have regained their former size and consistence. Subcutaneous injections of blood are not taken care of as readily as intramuscular injections and lead to extensive discoloration of the skin. We have noted once or twice a painful swelling of some of the injected muscles, which appeared at the end of ten days. This swelling rapidly subsided without any local treatment.

Twenty-three patients were treated at the Willard Parker Hospital with intramuscular injections of blood. They represent the cases with the poorest prognosis selected out of a total of some 900 admissions. Fifteen were treated with convalescent blood, which was citrated in a majority of cases. Nine received pooled blood from two or more donors. The amount injected varied from 2½ to 8 ounces. Of the 15 patients, 4 died; of these (a) one died from septic broncho-pneumonia two days after the injection; (b) one died of a streptococcus sepsis 9 days after the injection; (c) one was in extremis when injected intravenously; he seemed to rally slightly, but the treatment was evidently given at a time when the patient's nervous system was entirely overwhelmed by the toxemia; (d) the fourth case, practically moribund at time of injection had severe meningismus with a marked retraction of the head; he died 36 hours after injection. Of the 11 cases that recovered, only 5 were of the purely toxic type; the remaining six cases had additional severe septic complications, especially tonsillar and faucial exudates, and inflamed cervical glands. The 5 purely toxic cases showed a critical drop in temperature which varied from 3 to 6 degrees Fahrenheit, after which the temperature remained normal or slightly above normal. In the remaining 6 cases, the drop in temperature was less marked and was followed by a secondary rise which persisted for a number of days. These secondary temperatures were evidently due to the septic complications.

Eight patients received fresh normal blood; of this group none died. These cases were far advanced septic cases, several with a poor prognosis. The blood, which was readily obtained from one or both of the parents, was citrated in six. One of the patients received two injections of 6 ounces each; another three injections, each 8 ounces, at intervals of 4 to 5 days. As was to be expected, no striking critical drop, but a distinct lowering of the temperature curve was noted associated with an improvement in the septic complications and the general condition of the patient. The splendid results in some of these cases are the best proof of the beneficial effect that can be derived from this treatment.

While it is difficult to make any absolute statement or arrive at definite deductions from such a small series of cases, one can only hope that, by continuing this form of treatment, a larger series of cases may finally be collected that will serve as a more definite guide for the value of the intramuscular injections of convalescent and normal blood. The treatment is easily carried out, is quite harmless, and is within the reach of the general practitioner. The beneficial effects noted in the above group of very severe cases were quite distinct. The rational element in the use of convalescent blood is apparent, as long as the etiology of scarlet fever is not known, and the use of antistreptococcus serum during the past 10 years has not given the striking results that were expected from it after the early trials. The rational element in the use of fresh normal blood in the septic cases is found, first of all, in clinical observations of its great empiric value; but also to a large extent, in a consideration of the great nutritive value of homologous blood, its marked stimulating properties, and its well known antibody content, which exerts a distinct bactericidal effect on many pathogenic organisms.

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THE INTRAMUSCULAR INJECTION OF WHOLE BLOOD—ITS APPLICATION IN THE TREATMENT OF SCARLET FEVER AND OTHER CONDITIONS

Abraham Zingher

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During the past few years various methods have been elaborated for the transfer of human blood from donor to patient. At first defibrinated blood, and later fresh blood, was used. It is interesting to note that even twenty years ago von Ziemssen had employed with success a number of piston syringes and needles for the transfusion of fresh human blood into the vein of the recipient. Recently E. Lindeman[1] published a fairly simple and practical method for the transfusion of blood by means of syringes and special telescoping canulae. The method works well in his own hands, but has failed in those of a number of others, who either themselves lack the necessary skill or have not the skillful assistants needed for carrying out the transfusion. At least three such assistants and twelve or more Record syringes and special canulae are necessary.

In addition to these inherent technical difficulties there are the associated dangers of transfusion into the vascular system of patients, *i. e.,* the dangers from hemolysis and agglutination, from the injection of bloodclots and from overdistention of the patient's heart. Hemolysis is a real factor, as fatal cases of hemolytic jaundice and severe hemoglobinuria have been reported, when incompatible blood has been transfused. The danger from agglutination is less, although here too serious results may follow for the patient, especially when agglutination is associated with hemolysis. According to von Dungern and Hirschfeld human beings can be divided by their iso-agglutinins into four groups, the blood of the members of each group being compatible only with each other. This has often necessitated the exclusion of 9 out of 10 donors, because their blood was agglutinable or hemolytic, or both agglutinable and hemolytic with the recipient's blood. The blood tests often require from three to four hours for their completion.

During the past few years the advantages of fresh human blood in various pathological conditions have been repeatedly demonstrated, but the difficulties inherent in the old methods of using the canulae, and the new methods of using the syringes and canulae have deterred a great many from employing transfusion of blood, except in large centers and nearby sections, where skillful surgical help was within easy reach. During the last few months, however, a number of transfusions have been carried out by the citrate method, which is quite simple and requires less technical skill.

To avoid the difficulties of transfusion, especially where only the physiological properties of the serum are needed, a method is here presented for the intramuscular injection of fresh non-coagulated blood, either citrated or non-citrated, which is simple and easily carried out and involves no further skill than the ability to draw off a syringeful of blood from a large vein in the donor and inject it rapidly into the muscle of the patient.

The entire outfit consists of one or two 20 or 30 c.c. "Record" syringes and needles of steel or platinum-iridium (B. & S. gauge No. 16 or No. 17). These needles are short and of a convenient caliber, and allow a rapid entrance and exit of blood from the syringe.

Two all-glass 20 c.c. "Luer" or "Burroughs-Wellcome" syringes with steel of platinum-iridium needles will answer the purpose as well.

In an emergency any easy-working 10 or 20 c.c. syringe with a sharp, clean needle can be used.

As a tourniquet a small piece of rubber tubing and an artery forceps may be employed.

To carry out the transfer of blood, the donor is placed in a recumbent position and his veins at the bend of the elbow are made prominent by applying the tourniquet at the middle of the upper arm, care being taken not to obliterate the pulse. The skin is sterilized with alcohol and the median-cephalic or median-basilic vein is chosen. Places of injection on the recipient are also sterilized with alcohol or tr. iodin. As soon as the syringe is filled from the donor's vein, an assistant loosens the tourniquet and applies an alcohol sponge with slight pressure to the puncture opening. The operator immediately injects the blood into the muscle of recipient, inserting the needle almost perpendicularly to the skin in adults, but more obliquely in children on account of the thinness of the muscle. After the injection the syringe and needle are thoroughly washed in sterile normal saline, which is kept ready in a glass beaker. The entire time required for the aspiration of the blood and its injection need not exceed sixty seconds, a period which is far below the usual coagulation time of blood. The procedure is now repeated, using the other arm of the donor. For the third injection we go back to the arm originally used, and insert the needle through the same opening in the skin, being guided thereby into the original opening in the vein. We can thus avoid the additional pain from several skin punctures at different places, and also avoid injuring the vein any more than necessary. If care is taken, no hematoma will form, and the requisite amount of blood can be withdrawn from each vein. The blood can also be aspirated without repeatedly removing the needle from the donor's vein. An assistant steadies the needle in the donor's vein while the operator quickly detaches the full syringe and injects its contents into the recipient's muscle. During this interval the assistant keeps the needle free of blood by attaching a small Record syringe and injecting a few drops of normal saline or 1 per cent. citrate solution. This procedure is repeated until the required amount of blood is aspirated and injected.

The greater ease in using citrated whole blood has suggested its use in place of the fresh non-citrated blood. The necessary quantity of blood is aspirated from the median-cephalic vein of the donor and immediately citrated by adding in several 100 c.c. bottles the blood to a 10 per cent. solution of sodium citrate in the proportion of one ounce of blood to each c.c. of the citrate solution. The injection can then be carried out at the leisure of the operator.

The following muscles of the patient may be chosen and a syringeful of blood (20-30 c.c.) injected into each place:

Upper Extremities.—(1) Deltoids; (2) triceps.

Lower Extremities.—(3) Outer regions of the thighs, where the needle passes through the aponeurosis of the extensor fasciae femoris, and the blood is injected into the vastus externus; (4) the calves, where the blood is injected into the gastrocnemius and soleus; (5) the gluteal regions. If necessary, injections may also be made into the pectoral and interscapular muscles.

The injections are painless, except for the temporary slight discomfort associated with the entrance of the needle. No reaction follows, and the blood-mass appears to be rapidly absorbed, as indicated by the absence of tumefaction, pain or induration at the end of twelve to sixteen hours. The muscles will be found supple, and to have regained their former size and consistence. These observations can be made especially well in young children with thin muscles. If the blood is injected into the subcutaneous tissues, discoloration of the skin, and some infiltration will be noted in twenty-four hours, which will persist for a number of days.

Experimental work carried on in some of the lower animals has verified the above clinical observations. Guinea pigs and rabbits were injected subcutaneously and intramuscularly with varying quantities of homologous blood, citrated and non-citrated. When examined at the end of an hour the blood was found clotted; at the end of twenty-four hours the greater part of the blood-mass had become absorbed from the muscles, and only a part of the serum from the subcutaneous tissues. At the end of three to four days moderate quantities of blood had been completely absorbed from the muscle, while at the site of each subcutaneous injection a large coagulated mass of blood was still present.

A recent clinical experience in an anemic child (hemoglobin, 36 per cent.; red blood cells, 2,520,000), that had received 250 c.c. of blood into its muscles, showed that no immediate rise in hemoglobin and red blood cells took place. At the end of twenty-four hours the hemoglobin and red blood count were still unchanged. Whether the absorption of the blood-mass will finally result in an increase of hemoglobin and cells cannot be foretold; the fresh serum, however, with all its valuable antitoxic, antibacterial and ferment properties, is rapidly taken up, if the injections have been made intramuscularly, with little or no reaction on part of the surrounding tissues.

Meltzer and Auer[2] have shown that absorption from muscle occurs rapidly by way of the blood vessels, while from the subcutaneous tissues it is much slower and takes place by way of the lymphatics. According to these authors: "The effect of an intramuscular injection of adrenalin, curare, fluorescin or cocain stands nearest to that of an injection into the circulation and is far above that of a subcutaneous application."

These observations hold good also for colloids, as shown by Morgenroth and Levy.[3] They found that antitoxic serum is absorbed five to ten times faster from the muscle during the first three to six hours than from the subcutaneous tissues. These authors attribute the increased rapidity of absorption from the muscle to the richness of this tissue in blood vessels and also to the increased pressure within the muscle, which is absent in the loose subcutaneous tissue.

The ability to transfer fresh human blood in such a simple manner, and its assured absorption within a short time, would suggest a number of suitable conditions, in which the more difficult transfusion, or less efficient horse-serum are now used with advantage:

(1) In cases of hemorrhage, to stop persistent oozing by increasing the coagulability of the blood, *e. g.*, hemorrhage from the respiratory and gastrointestinal tracts, after tonsillectomy, in jaundice, in gastric and duodenal ulcers, in typhoid fever, etc. E. Weil has recommended the injection of fresh serum in cases of hemorrhage and it has proved of great value. Horse-serum has been used with success in some cases; in many it has failed, where blood transfusion was finally successful. The injection of heterologous sera (horse and rabbit) results in a local inflammatory reaction of the tissues which delays absorption and is probably the reason for some failures; in others, perhaps, the use of an old serum, where fresh serum was not available.

(2) In cases of hemophilia, the injection of fresh blood furnishes the necessary prothrombin. Repeated small transfusions have been recommended in these patients as a prophylactic measure.

(3) In purpura hemorrhagica.

(4) In certain chronic anemias: splenic and pernicious anemia, and the various leukemias. In these cases the donor's red blood cells after a blood transfusion probably do not remain very long in the patient's circulation.

In secondary acute anemias: after hemorrhage during major operations ruptured ectopic gestation, etc.

(5) In severe surgical shock.

(6) In acute and chronic forms of sepsis.

(7) In the malnutrition of infants.

(8) As a prophylactic procedure before various major operations.

(9) In various toxemias, especially scarlet fever. In this disease convalescent serum has been used with considerable success in selected cases of early very toxic scarlet.

With the exception, possibly, of the very acute surgical anemias, and some of the chronic blood diseases, the intramuscular injection of fresh human blood, as here described, may be found to have most of the advantages of direct blood transfusion without its well-known disadvantages. As a special instance, a case can be cited of typhoid fever, complicated with diphtheria, in which there was constant oozing from the nasopharyngeal tissues. The injection of 150 c.c. of fresh human blood into the muscles of the patient promptly stopped the oozing, which had not been influenced by a previous injection of horse-serum.

The donors chosen should be free from syphilis and tuberculosis, a Wassermann test excluding the former, and a physical examination the latter.

Intramuscular Injections of Whole Blood in the Treatment of Scarlet Fever

The method here described has suggested itself first in the treatment of the early very toxic forms of scarlet fever with fresh blood from convalescent patients.

Convalescent serum has been employed in small amounts for a number of years in the treatment of scarlet with varying degrees of success. It was not, however, until Reiss and Jungman,[4] in 1912, recommended the intravenous injection of larger quantities of serum (from 50 to 100 c.c.) that consistent results could be obtained. Rowe[5] has had a similar experience. More recently R. Koch[6] has again drawn attention to the therapeutic value of the antibody content of the blood-serum of convalescent scarlet cases taken from the eighteenth to the twenty-fourth day after the acute stage of the disease, and preserved with a small amount of phenol (0.5 per cent. of a 1:15 aqueous solution). The author reports observations on 22 cases of severe scarlet fever treated with the serum. He noted critical drops in temperature and improvement in the general condition of the patients; no effect, however, was observed on the septic complications, like joint, middle-ear and gland infections. In a few of the cases the intravenous injection of the serum has given rise to chills and symptoms of collapse. The author concludes that the serum deteriorates on keeping and will be found to have little or no therapeutic value after two to three months.

The intramuscular injection of fresh convalescent blood was carried out with rather striking results on two patients at the Willard Parker Hospital (service of Dr. R. S. Haynes), and they are, therefore, reported here in detail:

Case I.—E. G., nineteen years old, admitted to the hospital on December 20, 1914. History obtained from the father. Duration of illness twenty-four hours. Began with a sore throat, followed by a rash, on which the diagnosis of scarlet fever was made. Temperature on admission, 103.5° F.; slight delirium, intense purple rash, congested throat, no exudate, negative Schick test.

December 21st.—Temperature, 105° F.; delirium more marked, eyes congested, dusky rash over entire body. The same evening, 250 c.c. of fresh blood from two convalescent patients (thirty-one and thirty-seven days) injected into the deltoids, triceps, and outer regions of both thighs. After the injection the temperature showed a critical drop of 4° F., associated with a clearing up of the delirium, and a marked improvement in the general condition of the patient. Twenty-four hours later the temperature was normal and the patient made a rapid and uneventful recovery. Daily examination of the muscles at the sites of injection showed no local induration or tenderness.

Case II.—H. R., six years old, admitted to the hospital December 21, 1914. History of illness for three days before admission: General condition poor, intense dusky rash with small petechial hemorrhages. Cervical adenitis. Throat congested and swollen, greenish exudate on tonsils. (Culture negative for Klebs-Loeffler bacilli).

December 24, 1914.—On the third day after admission the patient appeared toxic and cyanosed. Delirium present, cervical glands large and tender, lips bleeding and ulcerated, throat congested and swollen. Condition considered partly septic and the prognosis seemed doubtful; 125 c.c. of convalescent blood (twenty-one days) was injected into the deltoids, triceps and muscles of thigh. During the night following the injection the temperature showed a critical drop of 4° F., followed by a temporary rise of 2° F. Within the next twenty-four hours the temperature became normal, and the general condition of the patient was considerably improved. Daily examination of the muscles at the sites of injection showed that the greater part of the blood had become absorbed within the first sixteen hours. The muscles remained supple and were neither infiltrated nor tender. On the left thigh the injection was made partly subcutaneous, and here a persistent slight infiltration and bluish discoloration of the skin were noticeable for a number of days.

The ease with which fresh convalescent blood from adult donors can be given by this method would suggest the possibility of its more frequent use, not only in early and very severe toxic scarlet fever cases, but also as a routine procedure in the moderately severe cases. In such patients, 100 to 125 c.c. of blood can be given to children, 200 to 250 c.c. to adults. These injections may prevent the toxic effects of the disease upon the heart, kidneys and nervous system of the patient; also, by increasing the resistance and diminishing the inflammatory condition in the throat, prevent the secondary septic manifestations in glands, middle ears and joints.

The adult donors should be tested with the Wassermann reaction and their consent obtained for the removal of 200 to 300 c.c. of blood. In a contagious disease hospital such donors are generally available. A record can be kept of their names and addresses, and, when required, either citrated, fresh convalescent blood from the donors, or the donors themselves, can be sent to the homes of private patients and the treatment thus made available in general practice.

SUMMARY AND CONCLUSIONS

(1) A simple syringe method is described for the intramuscular injection of fresh human blood in quantities of 125-250 c.c. at a sitting.

(2) The injection of homologous blood into muscle produces no inflammatory reaction and the resulting absorption is complete within three to four days, of the serum part alone probably in a few hours.

(3) The injection of homologous blood into subcutaneous tissue produces induration and discoloration of the skin, and the resulting absorption is delayed.

(4) The availability of the method would recommend its further trial in the treatment of scarlet fever and other conditions in which transfusion of blood and serum injections have been advised.

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A SIMPLE SYRINGE METHOD FOR THE TRANSFUSION OF CITRATED BLOOD IN CHILDREN

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The purpose of this paper is to present a simple method for the transfusion of citrated blood in young children. Transfusion has often been strongly indicated in a variety of medical and surgical conditions in these children, but the necessity for special skill in its performance, and the frequency of failure, have greatly limited its use.

The use of citrated blood in human transfusions was first practised in 1914 by Hustin, who injected in one case a mixture of citrated blood and glucose. Citrated blood in human transfusions was not used again, however, until R. Weil, in December, 1914, recommended the use of 1 per cent. sodium citrate. R. Lewisohn found that 0.2 per cent. sodium citrate will keep the blood fluid, and used that concentration successfully in a few human transfusions at Mt. Sinai Hospital. The method he employed for obtaining and citrating the blood consisted in allowing it to run from the vein through an ordinary cannula into a glass jar containing the proper amount of sodium citrate. The blood was then poured into an ordinary glass funnel and the usual intravenous infusion made. Frequently there is difficulty in obtaining the blood from the donor in this way on account of clotting from the slow flow of blood through the cannula. In the method described below the requisite amount of blood can be rapidly obtained, citrated in several small containers, and easily reinjected even into the small veins of young infants. The main part of the outfit consists of two Record syringes, two suitable needles and some sterile 10 per cent. solution of sodium citrate. In a series of experiments we were able to corroborate the statement that 0.2 per cent. of sodium citrate will keep human blood fluid, whereas 0.1 per cent. failed to prevent clotting. As an additional safety margin 0.33 per cent. is recommended.

It is interesting to note that R. Weil has injected as much as 5 grams of sodium citrate intravenously into adults, and the only effect noticeable was a transient polyuria. He also made the rather important observation that the sodium citrate temporarily hastened rather than delayed the coagulation of the blood.

Twelve transfusions were performed by us successfully on small children: eleven at the Willard Parker Hospital on the service of Dr. Louis Fischer, and one on a seven-weeks-old private patient of his. The median-cephalic vein in this infant, though very small, was readily exposed by means of a small incision in the skin, and the transfusion completed without difficulty.

The following technique is recommended:

Donor.—A suitable donor free from syphilis and tuberculosis is chosen. For small children one of the parents or some close relative will be found most suitable. The serum of the donor should not agglutinate or hemolyze the patient's red blood cells, or *vice versa.* This is conveniently determined by the method of Ottenberg and Epstein.

Instruments and Apparatus.—(*a*) One 30 c.c. and one 10 c.c. Record syringe.

(*b*) A platin-iridium or steel needle, 1½ inches long, No. 18 B. & S. gauge, with which the blood is obtained from the donor.

(*c*) A Goldenberg salvarsan needle (George Tiemann & Co.) for transfusing the blood into the patient.

(*d*) 6-12 sterilized wide-mouthed 100 c.c. bottles, in which the blood is collected from the donor.

(*e*) A number of sterile beakers or ordinary tumblers.

(*f*) A 10 per cent. and a 1 per cent. sterile solution of sodium citrate.

(*g*) A piece of rubber tubing and artery forceps as a tourniquet.

Obtaining the Blood from the Donor.—Into each of several 100 c.c. bottles we first introduce 2.0 c.c. of the 10 per cent. solution of sodium citrate. In a glass beaker some 1 per cent. solution of the citrate is kept ready for rinsing out the syringe.

The donor should be placed in a recumbent position and the tourniquet applied to the arm. The platinum needle, attached to the larger Record syringe, is now inserted into the tense median-cephalic vein, and a syringeful of blood quickly aspirated. An assistant now fixes the needle, while the operator quickly detaches the syringe and empties it into one of the 100 c.c. bottles. During this interval the assistant keeps the needle free of blood by attaching the smaller Record syringe and injecting a few drops of the 1 per cent. solution of sodium citrate. If two large Record syringes are kept on hand, the aspiration of the blood can become almost continuous. Experience has taught us, however, that even with a single syringe a large quantity of blood can be obtained within a few minutes. There is a decided advantage, also, in the intermittent withdrawal of blood, as it allows the donor's circulation to accommodate itself to the fairly rapid depletion, and avoids a sudden cerebral anemia.

Several syringefuls of blood can be readily obtained before it is necessary to rinse the syringe out with the 1 per cent. sodium citrate. Sixty c.c. of blood are put into each 100 c.c. bottle, making the final dilution of sodium citrate 0.33 per cent. It is important that with each addition of blood the entire contents be rapidly shaken, in order that the citrate may come into intimate contact with each amount of blood added.

Goldenberg salvarsan needle.

Within ten minutes from 8 to 16 ounces of blood, if needed, can thus be obtained, and is ready to be transfused into the patient.

Transfusion of the Blood.—The blood is aspirated from the bottles by means of a 25 or 50 c.c. pipette and is collected into a large beaker, or ordinary sterile glass tumbler. It is kept at body temperature by partly immersing the container in warm water. The top of the beaker is covered with a pad of sterile gauze. The best vein for the injection of blood is usually the median-cephalic. The next choice is the median-basilic or the external jugular. In older children the salvarsan needle can be inserted through the skin directly into the vein. In very young children, and especially in infants, it is preferable, and even necessary to expose the vein by a small incision, although occasionally one may be able to enter the external jugular vein without an incision. By exposing the vein, however, we are certain that the needle used for the transfusion is properly entering the vein, where it can be safely secured. A catgut or silk ligature is applied to the distal part of the vein; a similar ligature is slipped under the proximal part, but is not tied.

The large Record syringe which has been resterilized is now filled with blood from the beaker. If there is any tendency for the red cells to settle, the blood may be rapidly stirred with the end of the syringe. The Goldberg salvarsan needle is attached to the syringe, and carefully passed into the lumen of the vein, proximal to the tied ligature. This ligature serves to steady the vein, while the needle is being inserted. If care is taken, one can be successful even with the very small veins of young infants. The presence of the needle within the lumen of the vein is tested by first injecting a few drops of blood. If there is no leakage the assistant ties the upper ligature around vein and needle, and thus secures the latter in place. The blood is now slowly injected. As soon as the syringe is emptied, the assistant firmly fixes the needle, while the operator detaches the syringe and refills it with blood. The transfusion is continued until the required amount of blood has been injected.

It is important to be careful not to overburden the patient's circulation with a relatively large amount of blood, and it is therefore advisable not to inject more than 1 to 2½ ounces of blood in infants up to six months of age, 3 to 4 ounces up to one year, and 4 to 6 ounces up to two or three years of age. If necessary, the transfusion may be repeated at intervals of two to three weeks.

After finishing the transfusion, the needle is removed while the proximal ligature is firmly tied. The very slight wound is cleaned with sterile saline and the edges approximated with a strip of sterile adhesive plaster.

Where one desires to use non-citrated blood, the technique can be followed along very similar lines. Two assistants are needed. After securing the salvarsan needle in the patient's vein, we aspirate with a 20 c.c. Record syringe a syringeful of blood from the donor, detach the syringe and promptly inject the contents into the patient's vein. The needles in both donor and patient are kept free of blood by the assistants, who attach small (5 c.c.) Record syringes, filled with sterile normal saline and inject a few drops of the solution. The syringe used for the transfusion of blood is rinsed out with normal saline each time. If two syringes are used, the transfusion becomes almost continuous.

The ease and simplicity of the method would recommend its use in a variety of surgical and medical conditions. The large number of surgical indications need hardly be enumerated. The acute, subacute and chronic anemias, the hemorrhages in hemophilia and purpura, in jaundice, from gastric and duodenal ulcers, typhoid ulcers, etc., will readily suggest themselves. In the treatment of chronic primary anemias, as well as in marasmic and anemic children, successive small transfusions are often considered beneficial and may at times be life-saving. In the treatment of toxemias, like that of scarlet fever, transfusion is indicated, if a suitable convalescent donor can be found.

The outlook would be sufficiently hopeful, if a method for the transfusion of blood, which is easily carried out, could be placed in the hands of the general practitioner. The technique of the handling of syringes is easily acquired, and it should, no doubt, be taught to every medical student.

Conclusions.—1. A simple method is here presented for the transfusion of blood in young infants and children with an outfit which consists mainly of two Record syringes, two suitable needles and a sterile solution of sodium citrate.

2. The advantages claimed for the method are its simplicity, the rapidity with which the blood is obtained and citrated in several small containers, the complete control over the rapidity of the injection of the blood and the amount of blood injected, and the applicability of the method, even to very young infants.

BUBBLE FOUNTAIN TESTS

Jane L. Berry

The following tests were made upon material taken from the lip and bottom of a Bubble Fountain which was kept in the office of the proprietor for purposes of demonstration.

The fountain was of a well-known type, consisting of a porcelain cup attached to a water faucet midway between the main pipe and the faucet opening. The water enters at the bottom of the cup and forms a bubbling flow above the surface when the faucet is turned on, remaining level with the brim when the fountain is not in use. The force of the flow is not controlled automatically, but must be regulated by each user, or if desired the flow is directed through the faucet opening and not into the cup.

It was explained by the proprietor that an arrangement had been made by which the cup could be drained when not in use. This had been added to the design on account of the criticism that children and others might be tempted to drink by placing the lips at the edge of the full cup, but the emptying of the cup was not recommended by the proprietor; first, because the dry cup would accumulate dust, which might not readily wash out, and secondly, because the full cup is a necessary preliminary before the bubble effect can be obtained, and is the best safeguard against too strong a current upward through the narrow opening at the bottom when the water is first turned on.

The fountain is recommended on account of the simplicity of its construction and the ease with which it can be installed.

The material for cultures was taken directly after the fountain had been used for drinking, and was obtained by means of sterile swabs which were thoroughly rubbed over the surface of the lip and bottom of the cup, the swabs being then inserted into test tubes containing small amounts of sterile broth.

Test Specimen I was taken from the lip of the full cup, the swab having been first moistened with the water standing in the cup.

Test Specimen II was from the inside surface of the bottom of the cup after the water had been allowed to escape.

For Test III a sterilized swab was dipped in a broth culture of coli communis and then rubbed on the lip of the empty cup. After a few moments interval to allow of partial drying the faucet was turned on and the cup thoroughly flushed out, after which the lip was rubbed as above with a sterilized swab, which was placed in a test tube of sterile broth.

In Test IV the coli communis culture was applied to the inside surface at the bottom of the empty cup, and further as in Test III.

All specimens were at once taken to the laboratory, plated out on agar and the plates incubated at 37 degrees C. for twenty-four hours.

The five plates from Test Specimen I, containing altogether one-fourth of the entire amount of broth used in the experiment, showed but five colonies and a small conglomerate growth. All colonies showed staphylococcus albus.

Test Specimen II plated out in the same proportion, showed also five colonies, all staphyloccoccus albus.

Test Specimen III, in five plates, containing one one-hundredth each of the total amount of broth used, gave from thirteen to twenty colonies to each plate, all of the same gross appearance. The twenty colonies on one plate were fished and all showed coli communis.

Test Specimen IV, plated in the same proportion, gave from thirty-seven to forty-six colonies of the same general appearance in each plate. Of the thirty-eight colonies fished from one plate thirty-seven showed coli communis, and one, cocci.

Conclusions

The small number and innocent nature of the organisms found in the first two tests are arguments in favor of the use of this Bubble Fountain. On the other hand, the results obtained in Test III and Test IV constitute arguments against its use, as these results show what would be the danger to the public after accidental contamination of the fountain under ordinary circumstances. Since the organisms used were recovered in such large numbers after a more thorough flushing of the fountain than would ordinarily be applied, the danger becomes the more evident.

Some protection would be afforded through an automatic regulation of the force of the stream by means of which the bubble would always rise to the same height as soon as the faucet is turned on, thus providing against contact of the lips with the edges of the cup while drinking. Even if this precaution were carried out, however, there would still be no protection against contamination of the cup in the intervals of use, and the most effectual safeguard would be an arrangement providing for a continuous flow regulated at the proper height.

CIGAR CUTTER TESTS

JANE L. BERRY

There has been for some time more or less comment, both in medical and lay publications, upon the dangerous practice connected with the use of cigar cutters in tobacco stores. A man buys a cigar, places it between his lips, and then holding it in his mouth unlighted, proceeds to carry on a conversation with the proprietor or others. Finally, as he goes out, he lights the cigar after he has cut off the tip with the cigar cutter.

In this way, the machine is contaminated with the secretions of the mouth, which could readily be transferred to the cigar of the next customer.

In order to obtain more definite information with regard to this practice, an investigation was made, first as to the types of cigar cutters generally found in cigar stores; second, with regard to the use of the cutter by customers; and third, as to the presence of pathogenic organisms on the machine after use.

Inspections made by Mr. Levy showed that the cutters found in use, while differing considerably in design and appearance, could yet, in a general way, all be included under two main groups. In one group, there is an open knife blade attached to a shaft or frame work, upon which it moves up and down by means of a spring or lever, in the other group the knife is placed within a partially closed box-like apparatus, intended to catch the cut-off tips, and, on account of their shape, these are much more difficult to clean and more prone to harbor disease germs than cutters of the first type. Of twenty-five cigar stores visited in different parts of the city, nine had machines of the open type, and sixteen had various modifications of the closed box-like design.

With regard to usage of cigar cutters, careful observations were made in five stores with the following results:

1. *Upper 3rd Avenue*

Time—8.15 to 9.15 P. M.:

Tips bitten off	8
Tips cut dry	5
First held in mouth	0
Total customers	13

2. *Grand Street*

Time—7.30 to 9.25 A. M.:

Tips bitten off	10
Tips cut dry	9
First held in mouth	1
Total customers	20

3. *Delancey Street*

Time—8.45 to 9.25 P. M.:

Tips bitten off	15
Tips cut dry	15
First held in mouth	1
Total customers	31

4. *East Broadway*

Time—8.15 to 9.40 P. M.:

Tips bitten off	13
Tips cut dry	22
First held in mouth	2
Total customers	37

5. *West 42nd Street*

Time—9.45 to 11.00 P. M.:

Tips bitten off	24
Tips pinched off	5
Tips cut dry	15
First held in mouth	0
Total customers	44

Upon questioning proprietors as to the practice of cutting cigars wet with saliva, Mr. Levy was told that this was formerly done more frequently than at present, probably due, it was thought, to the increasing spread of hygienic ideas. At the same time, it was stated that the proportion of customers using the cutter in this manner was quite irregular, being sometimes much larger than would be indicated by the above figures.

Tests of bacterial contamination were made by a study of material taken by Mr. Levy from the cutters in use in two cigar stores, and also by means of experiments carried out with a cigar cutter brought to the laboratory for this purpose.

I

For the first test material was obtained at a store in East 14th Street from a cigar cutter of the closed type.

The control culture was taken from the cutter as found on entering the store at two o'clock P. M.

The test culture was taken from the same cutter immediately after it had been used to cut a cigar previously held in the mouth of the customer.

Each culture was made in three cubic centimeters of sterile ascitic broth by means of a sterile swab which had been moistened in the broth and then thoroughly rubbed over the lip and knife of the cutter. The swab was then placed in the tube of ascitic broth, and carried to the laboratory, where the broth was at once plated out, and the plates incubated at 37 degrees C. for 24 hours.

Plated in blood agar, the control culture showed no green or hemolyzing colonies. The test culture showed chiefly small green colonies with a few small hemolyzing colonies.

From the plates of the control culture, 20 fishings were made. All showed staphylococcus albus, generally pure, in some fishings mixed with rods.

From the plates of the test culture, there were 16 fishings. Three of these showed staphylococcus albus, thirteen showed streptococci in short chains. Of the latter, eleven strains were inoculated into white mice, but none proved to be pathogenic in a dose of one cubic centimeter.

COUNT OF PLATE COLONIES

	Control Culture	Test Culture
Plate 1 c. c.	300 colonies	2,000 colonies
Plate ½ c. c.	185 colonies	1,500 colonies

II

Material was obtained in Test II from the cigar cutter in a store in Grand Street.

The control culture was taken on entering the store at 7.30 A. M., the test culture was taken at 9.25 A. M. after a customer had used the cutter on a cigar wet with saliva from his mouth. The culture material was immediately brought to the laboratory and plated as in the first test.

No hemolysis and no green colonies appeared in blood agar plates from the control culture. The corresponding plates from the test culture showed a majority of small green colonies, no definitely hemolyzing colonies.

COUNT OF PLATE COLONIES

	Control Culture	Test Culture
Plate ½ c. c.	525 colonies	4,200 colonies
Plate 1/10 c. c.	135 colonies	2,000 colonies

Twelve fishings made from the control culture all showed staphylococcus albus.

Of eleven fishings from the test culture, three fishings showed staphylococcus albus, two fishings spore-bearing rods and six fishings non-hemolyzing streptococci.

III

Experiments have been made at the laboratory upon a cigar cutter of the closed box-like type by means of cultures made from the lip and knife before and after using the cutter in various ways.

In the first experiment a control culture was made as above described from the lip and knife of the cutter after it had stood for some days in the laboratory. Test culture 1 was then made in the same way after the cutter had been used on the end of a dry cigar. Test culture 2 was made after the cutter had been used on a cigar previously held in the mouth of one of the laboratory assistants. All cultures were immediately plated out.

The plates of the control culture and of test culture 1 showed no green or hemolyzing colonies in blood agar. The fishings showed staphylococcus albus. Blood agar plates of test culture 2 contained many small green colonies, also dense white growths. Fishings showed staphylococcus albus and spore-bearing rods. Streptococci were present, but could not be obtained in pure cultures.

Count of Plate Colonies

	Control Culture	Test Culture 1 (Dry Cigar)	Test Culture 2 (Mouth)
Plate ½ c. c.	8 colonies	440 colonies	800 colonies
Plate 1/10 c. c.	3 colonies	100 colonies	200 colonies

The next experiment was carried out by moistening the end of a cigar with a broth culture of pyocyaneus, then cutting the moistened tip, and taking a culture from the lip of the machine. As expected, the organism was recovered without difficulty.

A second experiment was made by cutting in the sterilized machine, a cigar moistened with pyocyaneus as above and then cutting a second cigar in the infected cutter. From this second cigar, culture of pyocyaneus were obtained by putting bits from the cut end into broth.

The cutter was next sterilized and taken to the Diphtheria Hospital, where cultures were taken from it after it had been used to cut cigars previously held in the mouths of patients with positive throats, the cultures being plated and fished as above. This experiment has been repeated three times. No Klebs Loeffler organisms were obtained from the cutter after it had been used on cigars held in the mouth by two of the patients, both in the active stage of the disease. When used on a cigar which had been held in the mouth of the third patient a culture obtained from the cutter showed Klebs Loeffler bacilli present. This patient was a convalescent only waiting for a negative throat report in order to be discharged from the hospital.

Conclusions

Since cigar cutters of the closed type are less readily cleaned and afford more harborage for bacteria, they would appear to be the more objectionable of the two main types in use.

A cigar cutter of any form for general use in cigar stores constitutes a serious menace to the public health on account of the more or less prevalent custom of using the machine upon cigars wet with saliva, the cigars having been previously held between the lips of the customers.

The posting of a notice warning against this practice attached to, or in close proximity with each cigar cutter, would no doubt be of decided value as an educative measure.

Since this warning would be disregarded in many cases, and as it would not be possible to maintain an adequate supervision, the most advisable measure with regard to the cutter in cigar stores would seem to be its abolition. The fact that 71, or nearly one-half, of the 145 customers observed made no use of the cutter would indicate that it is not an essential part of the cigar store equipment, and that no especial hardship would follow the passage of an ordinance forbidding the maintenance of cigar cutters for the use of the general public.

A STUDY OF THE COMMERCIAL PREPARATIONS OF BACILLUS BULGARICUS

ARTHUR J. BENDICK

(*Reprinted from Jour. A. M. A., March* 6, 1915. *Vol. LXIV, pp.* 809-10)

During the past few years many physicians have advocated the administration of living cultures of *Bacillus bulgaricus,* in order to change beneficially the intestinal flora. It is asserted that the large amount of acid produced by this organism from the fermentation of the various sugars is detrimental to the growth of other intestinal organisms, especially of the putrefactive group. This organism was at first given in the form of a fermented milk, but is now dispensed chiefly in the form of pure cultures, either in liquid or in tablet form. The potency of these cultures was the object of this investigation.

These cultures are sold either as liquid cultures—broth or whey—or in the dried form as tablets. The majority of the manufacturers simply label their product as living cultures of *Bacillus bulgaricus,* but do not state how many living organisms are present. Some state the number of bacilli present, but add that the count was made by some staining method (Wright), which method does not differentiate between living and dead organisms. As the therapeutic agent is the acid produced by the metabolism of the living organism, any count which does not differentiate between living and dead organisms is of no importance.

The *Bacillus bulgaricus* grows with difficulty on the usual laboratory mediums, but grows readily on mediums containing milk or whey. It ferments glucose and lactose readily, producing a large amount of acid, and the resulting acidity gradually kills the organism. It is this factor of acid production which is one of the principal causes of the rapid deterioration of the liquid preparations.

The names of the preparations tested are not given because not sufficient of each was tested to say which were uniformly bad or good. In the liquid preparations, those giving the hlghest counts were all obtained direct from local manufacturers from freshly prepared lots. The same preparations bought in the open market in other parts of the country would undoubtedly show much poorer results. In the more complete investigation which is being continued, I hope that results will be more definite, and that I shall be able to publish the names of those manufacturers who control their product so that it is uniformly good, and also to expose those whose preparations are practically inert or heavily contaminated.

In order to make an exact count of the number of living organisms in the preparation, it is necessary to plate them in suitable mediums in varying dilution, and after incubation to count the number of colonies. As the different strains of *Bacillus bulgaricus* vary in their cultural characteristics, each specimen was plated on four different mediums, and the highest count obtained on any one of the four mediums is the count that is listed. Plates were examined after three days' incubation at 40 c. (104 F.). The final count was made after five days' incubation.

The following plating mediums were used:

1. Veal infusion glucose agar.

To the veal infusion were added 2 per cent. agar, 2 per cent. glucose, 0.5 per cent. salt and 2 per cent. peptone, and the medium was titrated to 1 per cent. acid to phenol-phthalein.

2. Whey glucose agar.[1]

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[1] Heineman and Hefferan: A Study of Bacillus Bulgaricus, Jour. Infect. Dis., 1909, vi, 304.

Whey was obtained by adding glacial acetic acid to hot milk. To this were added 2 per cent. peptone, 2 per cent. agar and 1 per cent. glucose, and titrated to 1 per cent. acid.

3. Whey glucose calcium carbonate agar.[2]

This was the same as the previous medium, with the addition of a few drops of sterile powdered calcium carbonate in water just before plating.

4. Veal infusion whey glucose agar.

This was the same as the first medium, but after cooling to 45 C. (113 F.), one-fourth the volume of warm whey was added. This whey was prepared by rennet coagulation of certified milk. The coagulated milk was heated in the Arnold, and the whey thus obtained was filtered through paper and then sterilized.

Each specimen was also inoculated into the following tube mediums:

1. Litmus milk.
2. Whey.
3. Peptone glucose whey.
4. Veal infusion broth.

These tube inoculations served to control the plating mediums. In no case in which similar organisms were not obtained on one of the plating mediums was a growth obtained in any of the tubes.

The following specimens were tested:

1. Liquid. Obtained direct from manufacturer, freshly prepared lot. Broth culture in small bottles. Not dated. August 7, 1914, count showed 5,200,000 organisms per cubic centimeter. August 24, count showed 1,400,000. September 1, count 1,200,000. October 13, 1,200. October 30, specimens were sterile.

2. Liquid. Direct from manufacturer. Freshly prepared. Not dated. Direct plating, August 7, showed 40,000,000 per cubic centimeter, but by September 1, specimens were sterile on three separate examinations.

3. Liquid. Large bottle containing clear fluid with a heavy white precipitate which was readily miscible. Bought from retail druggist, November 24, 1914. Was dated with date of manufacture, October 24, 1914, with statement that it was good six weeks from date. Direct plating showed 5,600,000 per cubic centimeter. No contaminations. Organism grew vigorously and fermented dextrose rapidly. By December 9, the count had fallen to 1,700; December 31, to 124.

4. Liquid. Broth culture in small bottles. Bought from retail druggist, November 24, 1914. Expiration date, December 28, 1914. Immediate plating showed a count of 6 per cubic centimeter. December 7, specimen was sterile on three separate examinations.

5. Liquid. Bought direct from manufacturer, November 26, 1914. Expiration date, June 30, 1915. Broth in small bottles. Direct plating, one bottle gave a count of 12,400 per cubic centimeter, but another bottle gave a count of only 2 per cubic centimeter. December 31, 1914, one bottle gave a count of 95; another was sterile.

6. Liquid. Broth culture in small bottles. Left by demonstrator at physician's office. Expiration date, January 23, 1915. Direct plating, December 1, 1914, showed specimens sterile on three separate examinations.

7. Tablet. Bought from wholesale druggist, December 3, 1914. Expiration date, February 1, 1915. Tablets disintegrated rapidly in water, forming uniform mixture. Direct plating gave a count of 21,600 per tablet. December 15, a count of 10,200 was obtained.

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[2] Bendick: The Use of Calcium Carbonate in Solid Media for the Differentiation of Sugars Fermenting Bacteria, The Journal A. M. A., Oct. 21, 1911, p. 1343.

8. Tablet. Bought from retail druggist, December 9, 1914. Expiration date, April 23, 1915. Direct plating gave a count of 120 bacilli in a tablet.

9. Tablet. Bought from wholesale druggist, December 3, 1914. Expiration date, March 1, 1915. Plating showed the tablets to be loaded with contamination, a count of 59,200 other organisms being obtained. A moderate number of the *Bacillus bulgaricus* were present. Direct examination under the microscope of a tablet crushed in water showed the presence of numerous short motile bacilli, also a few long non-motile bacilli. Milk fermentation was atypical, digestion of the milk occurring with gas fermentation.

10. Tablet. Bought from retail druggist. Not dated. Told would keep indefinitely. Immediate plating showed numerous molds and a few short motile bacilli. On broth a heavy pellicle was obtained, and the milk was digested and gas formation occurred. No bacilli of the bulgaricus type were isolated.

11. Tablet. Bought from wholesale druggist, October 19, 1914. Expiration date, November 28, 1914. Immediate examination gave no growth on the plates, or in the milk, whey or broth.

12. Tablet. Obtained direct from manufacturer. Freshly prepared. One tablet gave a count of 105. Another 62. Three tablets were sterile.

Heineman and Hefferan[1] have demonstrated that *Bacillus bulgaricus* occurs normally in the feces of cows, horses and human beings; also in market milk and in soil. There is consequently no significance in the ingestion of a few bacilli in the commercial preparations. To be effective, the organisms should be present in large numbers. The commercial preparations should therefore be more carefully controlled, especially during transit and storage. I would suggest the following changes:

1. Preparations should be marked with the average number of living organisms to the cubic centimeter as determined by cultural tests, and also what percentage of these will be alive after definite intervals. This method would allow of accurate dosage, and give useful information. A count by the Wright method or a Gram stain is of no significance.

2. Preparations should be marked with the date of manufacture, with a statement of how long the preparation is potent. The present custom is to date with an expiration date, which method is a temptation to an unscrupulous dealer, and does not tell the age of the preparation.

3. Preparations should be kept in a suitable ice box as much as possible from the time they leave the manufacturer till they reach the patient. Cultures should therefore be obtained either direct from the manufacturer or from a few well-controlled supply depots.

CONCLUSIONS

1. The majority of the preparations of *Bacillus bulgaricus* contain only a small fraction of the number of living organisms that the manufacturers represent. Many of the preparations as sold at retail are sterile, containing no living bacilli of the bulgaricus type or any other living organism.

2. The majority of organisms as seen in a stained specimen are dead and hence are of no significance.

3. Broth cultures obtained direct from the manufacturers are the best preparations.

4. Tablets are useful if the product is to be conveyed a considerable distance. Some preparations of the tablets are potent, many are sterile, and some are heavily contaminated.

I desire to acknowledge my indebtedness to Dr. William H. Park, director of the laboratory of the New York Department of Health, for the material and for many valuable suggestions in the course of this work.

THE USE OF BRILLIANT GREEN FOR THE ISOLATION OF TYPHOID AND PARATYPHOID BACILLI FROM FECES

Charles Krumwiede, Jr., Josephine S. Pratt and Helen I. McWilliams

(*Reprinted from Jour. Infec. Dis., Vol.* 18, *No.* 1, *Jan.,* 1916)

While investigating the effect of various aniline dyes on the growth of bacteria[1] we found that brilliant green, aniline green, solid green, smaragd green, and china green, in appropriate dilutions, consistently restrained the growth of the gram-positive bacteria but varied in their action on the gram-negative bacteria. The most important variations were among the members of the colon-typhoid group. The growth of the typhoid bacillus was restrained but slightly, the paratyphoid-enteritidis types grew abundantly, whereas many of the colon group grew feebly or not at all. Many aerogenes types, as well as Bacillus proteus and Bacillus pyocyaneus, were unaffected, even when the dye was present in low dilutions. The concentrations at which this differential action was evident varied with the individual dyes. No one dye seemed more selective than another, but brilliant green exhibited the selective action at higher dilutions.

On the basis of these results, we suggested the use of brilliant-green broth for the enrichment of typhoid and paratyphoid bacilli in feces. Browning, Gilmour, and Mackie[2] suggested the same procedure for the enrichment of the typhoid bacillus, and Torrey,[3] for the enrichment of the paratyphoid-enteritidis types. The following is a report of the results of the application of this method and of further investigations of the action of brilliant green in fluid and solid media.

It had been found that the addition of feces to brilliant-green broth reduced the activity of the dye. It was necessary, therefore, in order to obtain the point of optimal differential action, either to use graded dilutions of the dye or to vary the amount of feces.

The method (Mr. Lawrence A. Kohn assisted us in carrying out the technical part of this work) employed in examining stools for typhoid bacilli was to add 0.1 c. c. of a moderately heavy suspension of feces to each of 3 tubes containing 10 c. c. of 1 per cent. glucose extract broth, neutral to phenolphthalein, to which brilliant green had been added to give final dilutions of 1 : 300,000, 1 : 400,000, and 1 : 500,000. After 15 to 18 hours' incubation, a loop from each tube was streaked on Endo plates, and these examined for typhoid colonies after incubation. Endo plates (Kendall's modification) were also inoculated directly from the suspension of feces used for the dye-broth tubes. The results obtained with this method in routine examinations for typhoid are given in Table 1.

TABLE 1

Results of Examination for Typhoid Bacilli in Feces in Brilliant-Green Broth

Number of Stools Examined	Result, Endo, Direct Inoculation	Result, Endo, Inoculation from Green-Dye-Broth
134	Negative	Negative
26	Positive	Positive
19	Positive	Negative
4	Negative	Positive
183		

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[1] Krumwiede and Pratt: Jour. Exper. Med., 1914, 19, pp. 20, 501.
[2] Jour. Hyg., 1913, 13, p. 335.
[3] Jour. Infect. Dis., 1913, 13, p. 263.

In the case of the stools giving positive results with both methods, there was, in some instances, a relative increase of the typhoid bacilli; in others there was a decrease. When stools were positive on direct plating and negative from the dye broth, the loss of the typhoid bacilli was due to the over-growth of dye-resistant organisms, especially the mucoid aerogenes types, and, less frequently, the paratyphoid-like intermediates or members of the proteus and pyocyaneus groups. The following experiment shows how easily such overgrowth may occur.

To 5 c. c. of a boiled suspension of feces were added 1 c. c. of a broth culture of typhoid and an equal volume of a broth culture of Bacillus [lactis] aerogenes. One-half cubic centimeter of this mixture was added to glucose broth containing 1:400,000 of brilliant green. This was plated, incubated, and plated at intervals, to determine the proportion between the two types. Plated immediately, B. aerogenes was to B. typhosus as 5 to 1; after 3 hours, as 9 to 1; and after 24 hours, as 300 to 1.

The work showed that a large proportion of the fecal flora could be suppressed. If this suppression could be obtained by a shorter exposure to the action of the dye, the subsequent loss of the typhoid bacillus by overgrowth of dye-resistant types might be avoided. In this way, the number of typhoid bacilli would be relatively increased although an actual enrichment due to multiplication would not occur.

Small amounts of suspensions of carrier stools (supplied to us by Dr. Anna M. Agnew of Long Island State Hospital) were added to suspensions of normal stools (to reduce the proportion of typhoid bacilli), and the resultant mixtures used for the following tests. After direct Endo plates had been made, 2 c. c. of the mixture were added to 4 c. c. of broth containing the dye. From this mixture, plates were made immediately and after 1, 3 and 18 hours, respectively. In some instances 2 dilutions of the dye, 1 : 100,000 and 1 : 300,000, were employed. No appreciable difference was noted. The results, with the use of a dilution of 1 : 300,000, are given in Table 2.

TABLE 2

Results of Examination for Typhoid Bacilli in Feces After Short Exposure to Brilliant-Green Dye

Number of Specimens	Direct Plates	Plates Made from the Dye Broth at Intervals.			
		Immediately	After 1 Hr.	After 3 Hr.	After 18 Hr.
1	Negative	Positive	Positive	Negative	Negative
1	Negative	Negative	Positive	Negative	Negative
1	Negative	Negative	Positive	Positive	Negative
1	Positive	Negative	Positive	Negative	Negative
7	Positive	Positive	Positive	Positive	Positive
3	Positive	Positive	Positive	Positive	Negative
Total 14	11 Positive 3 Negative	11 Positive 3 Negative	14 Positive 0 Negative	11 Positive 3 Negative	7 Positive 7 Negative
Average, percentage of typhoid on plates.....	50%	60%	63%	45%	2%

The shorter exposure to the action of the dye gave a greater increase of positive results over those in direct plating than had been obtained with the previous attempts at enrichment by longer periods of incubation. The growth on the plates showed, however, that this short exposure is not sufficient to prevent the growth of many of the dye-sensitive types present in the stool when they are subsequently transferred to a favorable medium.

The results with both methods were, on the whole, less satisfactory than had been anticipated. If a dye agar could be devised for direct plating, the suppression of the susceptible types would be more complete than when exposed for a short period to the action of the dye and then transferred to a favorable medium. Various combinations were tried but unsuccessfully, because the typhoid colonies were not sufficiently characteristic to allow of selection when other fecal types developed. It was thought at this time that an indicator was essential to differentiate the lactose-fermenting types. Many were tried but did not give sharp enough differences. The Endo indicator could not be used as the sodium sulfite reduces the activity of the brilliant green. The Andrade indicator, which consists of 100 c. c. of a 0.5 per cent. solution of acid fuchsin decolorized by the addition of 16 c. c. of a normal solution of sodium hydrate, was found to answer our purpose. The color of this indicator is restored by acids.

After many trials a medium of the following constitution was found to be satisfactory:

Liebig's extract of beef	3 grams
Witte's peptone	10 grams
Salt	5 grams
Agar	15 grams
Water	1,000 c. c.

Dissolve in the autoclave. The final reaction must be set to the Andrade indicator. This can be done at the time of preparation, or, as we have found more convenient, the agar can be rendered slightly alkaline to litmus and the final reaction set when used. If the reaction is to be set at once, 100 c. c. are placed in a bottle, 1 c. c. of the indicator added, and the whole titrated to a distinct red color, which will disappear on cooling. Time must elapse between the additions of acid or alkali, as the indicator adjusts itself slowly. The indicator is then added to the remainder of the agar, the reaction adjusted and finished in the ordinary manner. In the method we have employed, because of its greater convenience, the agar is rendered slightly alkaline to litmus, bottled in 100 c. c. amounts, and autoclaved. After determining the acid necessary by testing one bottle, this amount is added to the other bottles as used. This has the advantage that the reaction can be set more accurately in the clear agar and if the first bottle does not give completely satisfactory results, the amount can be varied. After addition of the acid the reaction to phenolphthalein (hot titration) is 0.6 per cent. to 0.7 per cent. acid.*

In either case, the indicator should be added and the reaction adjusted before the sugars and dye are introduced. To the melted agar, just before use, are added 1 per cent. of lactose and 0.1 per cent. of glucose—sterile 25 per cent. solutions of the sugars being used†—and, finally, the appropriate amount of a 0.1 per cent. solution of brilliant green. Each 100 c. c. will suffice for 6 plates, as the layer of agar must be relatively thick else the characteristic appearance of the typhoid colony is not as marked. The plates are allowed to stand open until the agar has solidified and then are covered, preferably with porous covers, to absorb the moisture. Inoculation is the same as with Endo plates.

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* Since the above was written we have found that the addition of 8 c. c. of normal soda per litre, after the ingredients are dissolved, gives a reaction in the stock agar that usually needs no correction. We have recently been using Fairchilds' peptone, but with a few trials the amount of soda necessary could be determined if other brands were used. As a rule, less dye is required with this method; see note under standardization.

† It is convenient to add both sugars in one solution, containing lactose 20%, dextrose 2%. Five c. c. per 100 c. c. of agar gives the requisite concentrations.

On this medium, many of the fecal types are restrained, whereas the typhoid bacillus produces large colonies which are very characteristic. Viewed through the plate against a dark background, with the light passing obliquely through the agar, they had a peculiar snowflake appearance. Seen by artificial light and a hand lens, under the same conditions. they have the appearance of a coarse wool fabric. They vary slightly in distinctiveness; as a rule, the larger colonies are the more typical and show best where the plates develop a moderate number of colonies. Other fecal types may develop typhoid-like colonies; but this similarity is usually no more troublesome than with Endo or other media.

To test this medium under the most natural conditions obtainable, we added to normal stools small traces of carrier stools. In this way, stools were made containing very few typhoid bacilli. The following results were obtained when suspensions of these mixtures were plated on Endo and on the green-dye agar:

Total examined	130
Endo agar	71 negative; 59, or 45%, positive.
Green-dye agar (0.2 c. c. of a 0.1% solution of dye to 100 c. c. of agar)	24 negative; 106, or 81%, positive.
Increased positive results	36%.

Of the 71 negative on Endo, 47 (or 67 per cent.) were positive on the green-dye agar. None was positive on Endo when negative on the dye agar.

Fifty-seven samples were also added to green-dye broth as in the preceding experiments, and, after one-half hour, plated on Endo. Of these, 8 were positive, although negative on the direct Endo plates, but 6 were negative which on the direct Endo plates had been positive. Twenty-two were negative which had been positive on the direct green-dye plates. None was positive which on the direct green-dye plates had been negative.

Sixty-six samples were enriched in the same way, but plated on green-dye agar instead of Endo. Three were negative which had been positive on the direct green-dye plates and 3 were positive which on the direct green-dye plates had been negative—as a result, in two instances, of the fact that the direct plates were overseeded.

At the same time 0.1 c. c. of the suspension was inoculated into 10 c. c. of glucose broth containing 1:300,000 of the dye, and after 18 hours' incubation plated on both Endo and green-dye agar. Forty-seven samples were tested. Six in both cases gave positive results which on the direct Endo plates had been negative, but 30 were negative which on the direct green-dye plates had been positive. In only one instance was the sample positive which on the direct green-dye plates, as well as on the Endo plates, had been negative.

While the comparative examinations described were being made, it was noted that with certain stools the number of colonies developing on the green-dye agar was only slightly less than on the Endo agar. Considerable difficulty was encountered in some instances because of the similarity of some of the colonies to those of the typhoid bacillus. These colonies were fished and found to be either intermediate types, slow lactose fermenters, or incompletely restrained colon types. It appeared to us that we might exclude these organisms with stronger dilutions of the dye. Although the number of typhoid colonies was decreased, they should be more easily found, were the typhoid-like type not present. Comparative tests showed that the concentration of the dye could not be carried much farther than 1:300,000, else the typhoid bacillus might not develop. From these tests it was decided to prepare the agar as before, but to use 2 series: one to contain 0.2 c. c., the other 0.3 c. c. of a 0.1 per cent. solution of the dye to 100 c. c. of the agar. In this series of comparative tests, Conradi's brilliant-green agar, of which we shall speak later, was also included. The material used for inoculation was a mixture of carrier and normal stools. The results are given in the following table:

TABLE 3

Results of Examination for Typhoid Bacilli in Feces Exposed to Different Concentrations of Brilliant Green

Result	Endo	Amount of 0.1% Dye Solution for 100 c. c. of Agar		Conradi
		0.2 c. c.	0.3 c. c.	
Negative	18	9	3	14
Positive	10	19	25	14
Average total of colonies developed	1,250	230	70	40
Average number of typhoid colonies	20	40	25	10

In one instance the agar containing 0.2 c. c. of the dye was positive, whereas the agar containing 0.3 c. c. was negative, because of almost complete inhibition of growth. In no instance were the Endo or the Conradi plates positive when our green-dye agar was negative. The figures given of the number of colonies developing on the different media are only roughly comparative, as more material was inoculated on the media containing the dye. They serve, however, to give an idea of the amount of restraint of the ordinary fecal types and the relative enrichment of the typhoid bacilli.

About this time a small, localized epidemic of typhoid occurring, we received 9 stools for diagnosis. Of these, none gave positive results on Endo medium, 4 were positive on the green-dye agar containing 0.2 c. c. of dye, and 5 were positive on the agar containing 0.3 c. c. of the dye. These natural stools acted as a control on the results obtained with normal stools inoculated with carrier stools.

The tests in this series were severe, as the stools were especially rich in dye-resistant types. When typhoid-like colonies developed on the agar containing less dye, they were in great part excluded on the agar containing more dye.

Table 4 gives the results of the routine examination of feces, chiefly from convalescents prior to discharge. This includes the specimens mentioned.

TABLE 4

Results of Routine Examination for Typhoid Bacilli in Feces

Number Examined	Endo	Green-Dye Agar	
		0.2 c. c.*	0.3 c. c.*
631	65 positive	89 positive 3 positive 7 negative	89 positive 3 negative 7 positive
		Total positive 99	

* Or its equivalent; see standardization.

In two instances the typhoid bacilli in the stools were unusually sensitive to the dye, as shown by the development of small to fine colonies. In spite of this, one of the samples was positive on the dye plates only.

In the use of the green-dye agar as described, many points have arisen as to the importance of the different constituents, which have been made clear by a number of comparative tests. We can best show these points by discussing each.

The concentration of the agar cannot be varied from that given without finding a new optimal concentration of the dye. Thus, if the dye be used with 3 per cent. agar, there is a marked decrease in its action.

The concentration of the peptone and meat extract has been found satisfactory, and no variations have been tried. By analogy with other observations, we are certain that any increase in the concentration will be accompanied by a decrease in the activity of the dye.

The trace of glucose was added because we had previously found that this amount of sugar would markedly increase the size of cholera colonies. As this amount of glucose is employed in the Russell medium, we did not anticipate that there would be sufficient acid produced by surface colonies to affect the indicator. The presence of this trace of glucose is the fundamental factor in the success of our medium. The typhoid colony is usually larger and more important; its distinctiveness is increased. Large colonies are desirable, as they furnish ample material for macroscopic slide agglutinations, which allow a rapid survey of many colonies. When the reaction of the medium is set close to the neutral point of the indicator, the use of glucose is followed by a slight tinging of the colony, which, however, greatly increases is characteristicity. Comparative tests without glucose have given very poor results because of the lessened distinctiveness of the typhoid colony.

The indicator has been tried in different concentrations in the hope of accentuating the lactose-fermenting types. On the whole, this has made no appreciable difference and we have decided to employ 1 per cent.

The lactose was added to differentiate the lactose-fermenting types. As many of them are inhibited, this is not so important as we at first considered it. Furthermore, most of the lactose-fermenting organisms which develop are either distinctive in their colony morphology (as the aerogenes types), or produce insufficient acid to affect the indicator. Similar results are noted in the use of the Endo agar, in which many colorless or nearly colorless colonies may develop, which, on isolation, are found to be lactose fermenter. On this account it might seem that the lactose and the indicator could be omitted, reliance being placed wholly on the distinctiveness of the typhoid colony. Such attempts, however, in many comparative tests, have shown very poor results. Many of the lactose-fermenting types simulate the colony of the typhoid bacillus so closely that great difficulty is encountered; this is not the case with plates inoculated with the same material, but with the indicator and the lactose present. As has been noted, if colon types develop partially on the dye agar, they will simulate the typhoid colonies. Similarly, many types which develop with a high dilution of the dye, but fail to develop at lower dilutions, show a typhoid-like colony. It would seem then, that the lactose aids in promoting the maximal development of those lactose-fermenting types which are able to develop at all. When this is the case, they do not resemble the typhoid colonies, although they may when their growth is restrained. This deduction seems warranted, as the greatest trouble is noted with the weaker dye agar, when lactose is omitted, whereas the troublesome types are excluded to a great extent on the stronger dye agar. As has been noted, the slight color developing in the typhoid colonies adds to their distinctiveness, but should it be absent, typhoid-like colonies are, as a rule, not confusing unless the lactose be omitted.

Some of the slow lactose-fermenting types which, under certain circumstances, develop typhoid-like colonies, change the indicator very slightly. This change, however, is sufficient to rob them of most of their typhoid-like appearance.

The reason for the 2 concentrations of the dye has already been fully discussed. The results show that both dilutions are necessary, as the stronger may give so great a degree of inhibition that the typhoid bacilli are lost.

One brand of brilliant green has been used in most of this work. Considerable variation is noted at times with certain solutions of the dye or certain batches of agar. A cloudy agar with sediment cannot be used, as the dye loses a large part of its activity. Different samples of the same brand of dye probably vary and the one brand of dye may not always be available. For this reason it was necessary to determine some method of standardization that could be used not only to test a sample of dye, but also to check the concentration of a dye solution with each batch of agar. When many routine examinations are being made, this takes comparatively little time, and the necessity of repeating the tests can be avoided by preparing the agar in large amounts.

Various methods for standardization were employed, using carrier stools, freshly isolated strains, as well as stock cultures of typhoid bacilli. As the amount of extraneous matter in the stools was a disturbing factor and the various strains showed some variations in resistance, the Rawlings strain, which is available in most laboratories, was finally adopted for the comparative tests. The amount of culture inoculated is important, as overseeding will obscure the gradations in the activity of the dye. Table 5 shows the results of a few standardization tests with varying amounts inoculated, these results serving as examples of the action of a correctly balanced dye agar

TABLE 5

RESULTS OF STANDARDIZATION TESTS

Endo	Amount of Dye to 100 c.c. Agar		
	0.2 c. c. Dye	0.3 c. c. Dye	0.5 c. c. Dye
Thousands	Thousands	Thousands	75% reduction
Thousands	Thousands	Slight reduction	90% reduction
2,000	2,000	1,500	No growth
200	200	180	No growth
60	60	50	No growth
40	40	35	No growth
30	25	6	No growth

In making the tests, the plates are streaked, but with a little practice fairly uniform inoculations can be obtained. In obtaining dilutions for inoculation, we have found that 0.5 c. c. of a broth culture diluted with 10 c. c. of broth will contain 2,000 to 5,000 bacilli per small loop. If a small loop of a broth culture be added to 10-15 c. c. of broth, a loop will contain from 25 to 200 bacilli.

If the dilutions given are tried, it is a simple matter to test out a sample of dye or a batch of agar. Should variations be noted from the results given, proportionate changes in the final dilutions of the dye to be employed can easily be determined. The fact that 2 dilutions are used compensates for slight variations.*

For an occasional examination with an untested sample of dye the use of 0.1, 0.2, 0.3 and 0.4 c. c. of the dye to 100 c. c. of agar would probably cover the range of action of most samples of brilliant green or variations in media.

Three samples of dye, from Bayer, Grübler, and Höchst, have been compared by this method, 2 solutions being used of each dye made from different parts of the sample. The variations between the different dyes were moderate and no greater than between the two solutions made from the same dye. With careful standardization it would seem, therefore, that any of these preparations could be used. If the test against the Rawlings strain gives correct results as far as we can see, the restraining action upon the fecal flora will be satisfactory. The degree of restraint of the fecal flora varies with different stools. The degree of restraint is, as a rule, greater with fresh specimens. In becoming familiar with the medium, we strongly advise the use of stools artificially inoculated with traces of carrier stools or with typhoid culture. The use of pure cultures may give misleading results, as fecal strains belonging to the B. coli group vary widely.

The dye solutions keep fairly well, but there seems to be some irregularity in their rate of deterioration. We have used some solutions as long as 2 months. It would be well, however, to retest a solution every 2 weeks as a safeguard against deterioration. The poured plates keep for several days. We have not tested this point as it is relatively unimportant to those doing routine examinations.

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* If a determined amount of alkali is added and no correction of reaction is made at the time of making the plates, as a rule less dye is required. The addition of acid apparently produces some precipitate, which decreases the activity of the dye.

Routine Method Now Employed

A large sample of the stool to be examined is rubbed up in extract broth and diluted with broth to a density (roughly a 1:15 dilution by volume of a formed stool) which, experience has shown, will give discrete colonies when a loopful is streaked over 2 successive Endo plates. The suspension is allowed to stand so that particles of feces will settle. One loop of the suspension is placed on a 0.2-c. c. and on a 0.3-c. c. plate; these are streaked in the order given and then an Endo plate. Two loops are placed on each of a similar pair of green-dye plates and streaked in the same order, and last an Endo plate. The wire used for streaking is not flamed while inoculating the series of plates. For practical routine examinations this seems a sufficient number of plates to employ. Although the Endo plates have never been positive when the dye agar was negative, we do not feel with our present experience that they can be omitted. Unusually sensitive strains are a possibility.

Some of the details of our technic might be described, as we find that they are not generally known. We do not employ glass rods to inoculate the plates, as they are unwieldy and cool too slowly after sterilization, but a suitably bent, heavy platinum wire with a loop at the end. A loop of the material is carried across the center of the plate and then smeared over the plate with the flat part of the wire, the plate being turned so that the entire surface is sown. A second plate can be inoculated in the same fashion without burning the wire. If 3 such wires are employed and used in rotation, they cool before being required again.

For a direct tentative agglutination from the plate, the macroscopic slide method is employed. A small drop of salt solution and one of an agglutinating serum are placed on a slide. The suspicious colony is taken up with a very small loop and rubbed first in the salt solution and then in the serum. A positive result is shown by immediate clumping, whereas the salt control remains evenly clouded. If spontaneous agglutination occurs, this will be noted in the control. A high titer serum is essential and the dilution must be lower than that ordinarily employed, as the reaction must take place very rapidly or the drop will dry. The optimal dilution must, of course, be determined for each sample of serum. As the fecal strains of typhoid are more agglutinable than blood strains, inagglutinable strains are not so apt to be encountered. One peculiar source of error has been noted, however. Certain strains have shown a prezone phenomenon; that is, they agglutinated poorly or not at all at low dilutions, but agglutinated well at higher dilutions. However, this method, with a known serum, gives results that are very reliable and we give a tentative diagnosis on this basis. Unknown sera, especially if they be fresh, may lead to serious error unless thoroughly tested with various types developing on the plates, as well as with known strains of allied bacilli.

For fishing, the Russell medium is employed with the following modification. In place of litmus, 1 per cent. of the Andrade indicator is used, and the reaction set accordingly. This modified medium gives much sharper results, and the acid production in the butt is very evident; the indicator does not decolorize, as is the case with litmus. As the medium is rather delicate, each new lot should be tested, before use, with a few known strains, to be sure that it is not too sensitive.

In fishing from the green-dye agar there is the possibility of not obtaining pure cultures. This is a disadvantage of all differential restraining media, the restrained bacteria being able to grow when transferred to a favorable medium. It is well to save suspicious plates until the fishings are inspected, or to replate from them immediately.

Besides inoculating plates in the manner described, 0.1 c. c. of the fecal suspension may be added to 1 per cent. glucose extract broth, neutral to phenolphthalein containing 1:300,000 of the dye. If, as occasionally happens, very little growth develops on the direct dye agar and no typhoid is found, Endo agar is inoculated from the broth tube after 18 hours' incubation. This is most likely to be successful when the flora is dye-susceptible, giving an added chance of success. Should the dye agar, however, show abundant growth, sub-inoculations from the dye-broth tube seem superfluous.

There would seem to be no reason why these results cannot be applied to the isolation of members of the paratyphoid-enteritidis groups. We have had only one naturally infected specimen,—a sample of soup which produced gastro-enteritis in several members of a family. When this was plated on Endo, about 1,000 colonies developed, of which 1 was a paratyphoid type. On green-dye plates of the usual strength, only a few paratyphoid types developed.

Pure cultures of this group were plated with seeding well under 150 for a plate, to determine whether appreciable restraint would be evident on the green-dye agar of 0.2, 0.3 and 0.5 c. c. strength.

Most of the strains showed no, or at most slight, inhibition on the strongest dye agar. Some showed a moderately marked inhibition, and a few failed to grow on the 0.3 c. c. dye agar. A few showed inhibition even on the 0.2 c. c. dye agar. This agrees with our previous conclusion[2] that the members of this group have a variable sensitiveness to brilliant green, although most of the strains are extremely dye-resistant.

The colony morphology is variable. The more feebly growing types are typhoid-like; the more vigorous strains produce 2 kinds of colonies with intermediate gradations. Both types are larger than the typhoid colony. One type of colony is yellowish-white, raised, and moist. The denser center is homogeneous, but the edge shows some markings similar to those in the typhoid colony. The other type is flattened, spreading, feathery, and irregular in outline, the typhoid-like markings being exaggerated. Some strains produced both types of colonies, and fishings on replating bred true.

The use of dye broth for enrichment is more likely to be successful with the resistant members of this group, as stronger dilutions of the dye may be employed.[2]

We have not discussed the results of previous investigations of the application of aniline dyes for the enrichment of the typhoid-paratyphoid group. Our own work developed independently on the basis of our observations of the action of the various aniline dyes. As the outcome has shown, it is fortunate that we did not attempt merely to modify existing media. We have tested several of the methods suggested, with results inferior to our own. No attempt was made to try out the various malachite-green media, as our results have shown that this dye is inferior to brilliant green in differential action. Furthermore, different samples of malachite green have been found to vary widely in their action, whereas, most samples of brilliant green give similar results.

As the Conradi brilliant-green agar[4] approached most nearly the medium we have devised, we have employed it in comparative tests. The results, given in the preceding tables, are not very favorable to Conradi medium. Furthermore, this latter, containing 3 per cent. of agar (which seems to us unnecessary), and 20 grams of meat extract to the liter (whereas we used only 3), is more difficult to make and more expensive—both factors of importance in routine work. On the whole the Conradi medium is too restraining; the colonies are somewhat characteristic but insufficiently so. German workers agree with us in these points. The Conradi agar is now chiefly employed as a preliminary enrichment medium. The plates are heavily inoculated and after incubation are flooded with broth, whereupon the typhoid colonies, less adherent than other types, float off easily, and the suspension is inoculated either on Conradi-Drigalsky medium or Endo. We have tried this method with different green-dye media, and although the results were better than from the use of Endo medium and the usual technic, they were not as good as with our medium alone. Furthermore, this method delays the results one day.

China green has been employed by Werbitski, and some workers have found it superior to the Conradi brilliant-green medium as a preliminary enrichment medium. We did not find China green more differential in its action than brilliant green; moreover, its action is not evident at equally high dilutions. For this reason, we have not tried this medium and are inclined to attribute any better results obtained in its use as referable to a better adjustment of the dilution of the dye to the other constituents rather than to any inherent superiority in the dye itself.

The attempts of various workers[4] to use a differentially selective dye decolorized by sodium sulfite and designed to act both as a restraining agent and indicator, are, in our opinion, due to a misconception of the mode of action of the dyes employed. We have found it impossible to balance the degree of loss of action of the dye due to sodium sulfite. We have also employed caffein, as advised by some investigators, with only moderate success.

It would require too much space to analyze even a small part of the literature which exists on typhoid isolation. The mere existence of a great number of methods is evidence of the unsatisfactory results obtained. On the whole, however, the application of the selective media described in this paper has been very successful in the isolation of the more resistant paratyphoid-enteritidis group.

SUMMARY

With an easily prepared agar, 2 concentrations of brilliant green being used, it has been possible markedly to increase the per cent. of positive typhoid isolations from stools. This has given much better results than the employment of brilliant-green broth alone, although the dye-broth enrichment method may give positive results when the agar alone fails. The methods can be applied to the isolation of the members of the paratyphoids enteritidis group from stools.

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[4] Kutscher: Kolle and Wassermann, Handb. d. pathogen. Mikroorganismen, 1913, 13, p. 717.

FURTHER OBSERVATIONS ON THE GROWTH OF BACTERIA ON MEDIA CONTAINING VARIOUS ANILIN DYES, WITH SPECIAL REFERENCE TO AN ENRICHMENT METHOD FOR TYPHOID AND PARATYPHOID BACILLI

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In a previous communication (1) we reported the results of a series of observations on the growth of thirty varieties of bacteria on media containing various dyes. We have continued this work, using the same series of cultures and forty samples of dyes. The methods employed have been the same. Green dyes are widely used in special media for typhoid and paratyphoid isolation. This led us to include many of these dyes in our present work, in the hope that some might be more selective than those in use. If this were the case, their application to plate and fluid media would be of great value.

In general, the results have been similar to those with other dyes, especially gentian violet and its allies (Table I). The inhibition of growth has been most evident among the Gram-positive bacteria, the Gram-negative bacteria as a rule growing freely on the dyed agar.

Certain exceptions are evident in the table. The cholera strain employed was less resistant to the action of green dyes than the allied vibrios. To determine whether this was of differential value, forty cultures of cholera and other vibrios were tried on agar containing *Bittermandelölgrun,* the dye showing the greatest differences. A variable restraining action was noted, but there were no differences according to types.

The diphtheria bacillus and its allies have given apparent differences and irregularities. This is due to the tendency of these cultures to produce flakes which, when inoculated on the agar, produce a limited growth, whereas the separate bacilli fail to grow.

TABLE I[1,2]

	Bacteria Tested	Brilliant Green, Crystals 366 (Bayer)			Brilliant Green			Anilin Green			Solid Green			Victoria Green, Crystals 4,833 (Bayer)			Smaragd Green			China Green			Malachite Green Crystals, "Chlorzink-doppelsalz"			Bittermandelolgrun			Victoria Blue 4 R.			Malachite Green			Malachite Green (Hochst)		
		1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000
Gram-positive	Staphylococcus aureus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	*	—	—	×	—	—	*	—	—	*	—	—	—	—	—	×	—	—	×	—	—
	Streptococcus (green)	—	—	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	*	—	—	±	—	—	±	—	—	×	—	—	×	—	—
	Streptococcus (hemolytic)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—	×	—	—	×	—	—	—	—	—	—	—	—
	Streptococcus mucosus capsulatus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	×	—	—	*	—	—	—	—	—
	Pneumococcus	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—	×	—	—	—	—	—	*	—	—	*	—	—	—	—	—	—	—	—
	B. diphtheriae	×	—	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—	×	—	—	×	—	—	×	—	—	×	×	—
	Diphtheroid bacilli (Cameron)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	×	—	—	×	—	—	×	—	—
	Diphtheroid bacilli (Cunningham)	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	±	—	—	×	—	—	×	—	—
	B. hoffmanni	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	±	—	—	±	—	—	×	*	—	×	—	—
	B. subtilis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	×	—	—
	B. lactimorbi	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	*	—	—
	B. anthracis	—	—	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	*	—	—	±	—	—
Gram-negative	Cholera	*	—	—	*	—	—	—	—	—	—	—	—	±	—	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	+	×	*	+	+	×
	Vibrio (indol-positive)	*	—	—	×	*	—	×	—	—	—	—	—	±	×	*	×	×	—	+	×	—	+	±	×	+	±	×	+	+	±	+	+	±	+	+	+
	Vibrio (indol-negative)	±	—	—	+	×	—	+	—	—	±	×	—	±	*	*	±	×	*	+	±	*	+	×	*	+	±	—	+	±	—	+	+	±	+	+	+
	B. proteus	±	*	*	+	×	*	+	*	*	±	×	—	+	±	×	+	×	*	+	±	×	+	±	±	+	±	×	+	±	×	+	+	+	+	+	+
	B. pyocyaneus	+	+	+	+	+	+	+	+	+	+	+	+	+	±	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriae (Shiga)	×	—	—	±	×	—	±	—	—	+	*	—	+	—	—	+	*	—	+	*	—	+	—	—	+	—	—	+	+	+	+	±	*	+	+	+
	B. dysenteriae (Flexner)	—	—	—	×	×	—	—	—	—	—	—	—	+	—	—	×	—	—	+	*	—	+	—	—	+	—	—	+	+	+	+	±	*	+	±	×
	B. dysenteriae (Mt. Desert)	×	—	—	×	×	—	×	—	—	×	—	—	+	—	—	×	—	—	+	*	—	+	—	—	+	—	—	+	+	±	+	±	*	+	±	±
	B. typhosus	+	×	—	+	×	×	+	×	—	+	×	—	+	+	×	+	×	×	+	+	×	+	±	±	+	+	±	+	+	+	+	+	+	+	+	+
	B. paratyphosus, A	+	+	±	+	+	±	+	+	±	+	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, B	+	+	±	+	+	±	+	+	±	+	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. enteritidis (Gaertner)	+	+	+	+	+	±	+	+	±	+	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Bacteria Tested	Brilliant Green, Crystals 366 (Bayer)			Brilliant Green			Anilin Green			Solid Green			Victoria Green, Crystals 4,833 (Bayer)			Smaragd Green			China Green			Malachite Green Crystals, "Chlorzink-doppelsalz"			Bitter-mandel-olgrun			Victoria Blue 4 R.			Malachite Green			Malachite Green (Hochst)		
		1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000
Gram-negative	B. coli communis	±	*	—	+	×	—	+	—	—	+	*	—	+	±	—	±	×	—	+	+	×	+	±	±	+	+	±	+	+	±	+	+	+	+	+	+
	B. coli communior	±	—	—	+	×	—	+	—	—	+	×	—	+	±	—	±	×	—	+	+	×	+	±	±	+	+	×	+	+	±	+	+	+	+	+	+
	Friedlander's bacillus	±	*	*	+	±	×	+	×	×	+	±	×	+	±	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. lactis aerogenes	×	—	—	+	×	*	+	—	—	±	×	—	+	±	*	±	±	×	+	+	×	+	+	+	+	+	×	+	+	±	+	+	+	+	+	+
	B. ozaenae	—	—	—	×	—	—	×	—	—	×	—	—	+	*	—	×	*	—	+	±	×	+	±	±	+	±	*	+	+	±	+	+	+	+	+	+
	B. pullorum	+	±	±	+	±	—	+	+	±	+	+	±	+	+	±	+	+	±	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+

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[1] In the tables +=growth like control; ±=restrained growth; ×=markedly restrained growth; —=no growth; *=some growth developed later; §=several colonies.

[2] In addition to the dyes given in the table, the following were also tested: alkali green, *Säurealizaringrün*, *Lichtgrün* F. S., *Brillantblau (extra grünlich)*, naphtol green B., *Echtgrün*, *Säuregrün*, alizarin green B., methyl blue, trypan blue, anilin blue (water-soluble), China blue, anilin blue-black, *Blauschwarz* and *Wasserblau*. In all the dilutions employed growth was as vigorous as on the control. Where not otherwise stated, all the dyes employed were obtained from Grübler.

TABLE I—*Concluded*

	Bacteria Tested	Malachite Green Ia			Nachtblau			Methylene Blue, med. Pure			Janus Green			Magenta Red			Methyl Green, 00 Rein			Chlorhydrin Blue, or Acetin Blue			Pyronin			Iodin Green			Alkali Blue			Methylene Green			Methyl Green		
		1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000	1: 500,000	1: 100,000	1: 50,000
Gram-positive	Staphylococcus aureus	×	−	−	±	−	−	±	×	*	+	*	−	+	±	×	±	±	*	+	+	+	+	+	+	+	±	×	+	+	+	+	+	+	+	+	×
	Streptococcus (green)	±	−	−	±	−	−	+	−	−	+	×	*	+	±	×	+	+	+	+	+	+	+	×	−	+	+	+	+	+	+	+	+	−	+	+	+
	Streptococcus (hemolytic)	−	−	−	±	−	−	+	*	−	+	×	−	±	×	−	+	+	+	+	+	+	+	*	−	+	+	+	+	+	±	+	±	*	+	+	±
	Streptococcus mucosus capsulatus	*	−	−	×	−	−	+	−	−	+	×	−	+	±	×	+	+	+	+	+	+	+	×	−	+	+	+	+	+	±	+	+	±	+	+	+
	Pneumococcus	−	−	−	±	−	−	+	−	−	+	±	−	+	±	±	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	×	+	+	±
	B. diphtheriæ	±	×	−	±	−	−	×	*	−	+	−	−	×	−	−	±	×	−	×	×	−	+	+	±	+	±	×	+	±	×	+	+	±	+	+	±
	Diphtheroid bacilli (Cameron)	×	−	−	−	−	−	−	−	−	+	−	−	+	−	−	×	−	−	×	−	−	+	+	±	+	×	×	+	×	×	+	+	±	+	+	±
	Diphtheroid bacilli (Cunningham)	×	−	−	−	−	−	−	−	−	−	−	−	×	−	−	±	*	−	×	−	−	+	+	+	+	±	×	+	×	×	+	+	±	+	±	×
	B. hoffmanni	×	−	−	−	−	−	−	−	−	−	−	−	×	−	−	+	*	−	×	−	−	+	+	+	+	±	×	±	×	×	+	+	*	+	±	×
	B. subtilis	×	−	−	±	−	−	±	±	×	+	−	−	+	×	−	+	×	−	+	+	+	+	+	+	+	±	*	+	+	+	+	+	+	+	+	×
	B. lactimorbi	*	−	−	×	−	−	±	*	−	±	−	−	+	×	−	+	±	*	+	+	+	+	+	+	+	±	±	+	+	+	+	+	×	+	+	×
	B. anthracis	×	−	−	±	−	−	+	+	±	+	−	−	+	±	×	+	±	−	+	+	+	+	+	+	+	±	×	+	+	+	+	+	+	+	+	±
Gram-negative	Cholera	+	+	×	+	+	+	+	×	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	×	+	+	+
	Vibrio (indol-positive)	+	+	+	+	+	+	+	×	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Bibrio (indol-negative)	+	+	+	+	+	+	±	±	×	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. proteus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. pyocyaneus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Shiga)	+	+	+	+	+	+	+	±	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Flexner)	+	±	×	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Mt. Desert)	+	±	×	+	+	+	+	+	±	+	±	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. typhosus	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, B	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. enteritidis (Gaertner)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communior	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Friedländer's bacillus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. lactis aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. ozænæ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. pullorum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

With one exception the results obtained have failed to show variations which could be used for practical differentiation. They are given, however, as similar detailed observations are not available in the literature.

The observations on the green dyes show marked variations in the resistance of members of the typhoid-paratyphoid-colon group. In general the paratyphoid-enteritidis types are highly resistant, the typhoid less so, but somewhat more resistant than the coli types. Various green dyes (2) have been employed in media to suppress the colon types found in feces. Loeffler employed malachite green agar, Werbitzki China green, and Conradi brilliant green agar, adding picric acid, which reduces the activity of the dye. The media have been of some use, especially for preliminary plating for enrichment, for paratyphoid more than for typhoid. On these media the difference in resistance of the typhoid and colon types was slight. If very few typhoid bacilli were present or if less resistant than the average, failure resulted. Peabody and Pratt and others attempted to use malachite green in fluid media as an enrichment medium.

Of the dyes we tried, ten revealed variations among the typhoid-colon group. A large number of cultures of the group were obtained[3] and grouped according to their sugar fermentations, and tried on agar containing some of these dyes. This was done to determine whether one dye showed wider differences than another and as a preliminary to the application of the results already obtained, to a selective plating medium, or a fluid enrichment medium. Agar is an easier medium to employ as an index, and was therefore used, as we had found that the results on agar could be duplicated in broth with slight variations in dilutions. To reveal slight differences in reaction, a small number of bacteria were inoculated. A loopful of a broth culture was carried to a second tube and loop inoculations were made from this (Table II).

The results show that the different dyes possess a similar differential action. This difference, however, appears at different dilutions according to the dye employed. At the appropriate dilutions no one dye is perceptibly more differential than another.

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[3] We are indebted to Dr. Torrey and Prof. C.-E. A. Winslow for some of the cultures employed.

TABLE II[4,5]

Type Tested	No. of Strains	Glucose	Dulcite	Lactose	Raffinose	Saccharose	Anilin Green			Brilliant Green (Grübler)			Brilliant Green (Bayer)			Smaragd Green			Solid Green			Victoria Green (Bayer)			China Green		
							1: 300,000	1: 100,000	1: 10,000	1: 300,000	1: 100,000	1: 10,000	1: 300,000	1: 100,000	1: 50,000	1: 100,000	1: 50,000	1: 10,000	1: 100,000	1: 50,000	1: 10,000	1: 100,000	1: 50,000	1: 10,000	1: 100,000	1: 50,000	1: 10,000
B. alkaligenes	2	—	—	—	—	—	—	—	—	—	—	—	..	..	..	—	—	—	—	—	—	..	..	..	..	..	..
B. typhosus	3	+	—	—	—	—	±	*	—	×	*	—	±	*	—	—	—	—	×	—	—	±	×	*	±	×	×
B. paratyphosus, A	6	†	†	—	—	—	+	±	×	+	+	×	+	±	×	±	±	×	+	+	×	+	+	+	+	+	+
							±	*	—	±	×	—	±	×	*	—	—	—	§	—	—	±	±	—	+	±	±
B. paratyphosus, B	4	†	†	—	—	—	+	+	±	+	+	±	+	+	±	±	±	±	+	±	±	+	+	+	+	+	+
							+	±	—	+	±	—	±	±	×	×	*	—	±	×	*	+	+	±	+	+	±
B. enteritidis	2	†	†	—	—	—	+	+	×	+	±	×	+	±	±	+	±	×	±	±	×	+	+	+	+	+	±
							+	±	—	+	±	—	±	×	—	×	*	—	±	±	—	+	+	+	+	+	±
Intermediate types	4	†	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	±	±	×
	1	†	—	—	+	—	—	—	—	—	—	—	..	..	..	—	—	—	—	—	—	..	..	..	..	..	..
	1	†	†	+	†	†	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—	±	×	§	+	±	×
	1	†	†	+	†	+	—	—	—	—	—	—	..	..	..	—	—	—	—	—	—	..	..	..	..	..	..
B. acidi lactici	6	†	—	†	—	—	—	—	—	—	—	—	..	..	..	—	—	—	—	—	—	..	..	..	..	..	..
B. coli communis	3	†	†	†	† or —	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B. coli communior	6	†	†	†	†	†	+	+	×	+	±	±	+	±	±	±	×	×	±	±	×	+	+	+	+	+	+
							—	—	—	—	—	—	×	—	—	—	—	—	—	—	—	×	*	—	±	×	*
B. aerogenes types	6	†	—	†	†	†	+	+	±	+	±	±	+	+	±	±	±	±	+	±	±	+	+	+	+	+	+
							—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[4] Where two sets of observations are given they are the extremes on that type of culture.

[5] —=no fermentation; +=acid production; †=production of acid and gas; ..=no observations. Other symbols are as in preceding table.

Five cultures were not included in the preceding table. These are the cultures used by Churchman and Michael (3). We have taken their observations on the growth of these cultures on gentian violet agar and their interagglutinations, and added our observations of the sugar fermentations of the same cultures and of the growth on media containing green dyes. Table III shows the possible application of dye media to the differentiation of some members of the enteritidis group. The question that immediately arises is: Has the reaction to dyes any relation to their source or pathogenicity? For instance, two cultures, E 25 and E 26, were isolated from a drainage canal by Jordan. Unfortunately the source of the other cultures is not available. If the strains which did not grow came from human beings suffering from infection by these organisms, there will be times when the application of these dyes for enrichment methods will fail.

TABLE III

Organism	Growth on Gentian Violet Agar	Growth on Agar, or in Broth with Green Dyes[6]	Glucose	Dulcite	Summary of Agglutination Reactions			
					Sera			
					E 18	E 25	E 26	E J.H.H., Same as E 234
E 18 (67)........	+	+	†	†	+++	o	o	++
E 25 (68)........	+	—	†	—	o	+++	o	o
E 26 (14).......	+	—[7]	†	†	o	o	+++	o
E 132 (70).......	+	+	†	†	+++	o	o	++
E 234 (64)	—	—[7]	†	†	++	o	o	+++

[6] Dyes given in Table II.
[7] Very slight delayed growth on agar containing 1 : 500,000 of some of the dyes.

Experiments were then begun with fluid and solid media for differential enrichment. The use of solid media was not as successful as fluid media. As we had not found any one dye more selective than another and because of two reports giving results similar to our own which appeared at this time, we continued to work with brilliant green.

Torrey (4) found that by the use of Grübler's brilliant green in fluid media he could enrich the paratyphoid-enteritidis group and restrain the common fecal bacteria. Browning, Gilmour and Mackie (5) were able, by the use of Bayer's brilliant green in fluid media, to isolate typhoid or paratyphoid bacilli from stools, where direct plating was unsuccessful.

Preliminary tests were made to determine what medium to use. Peptone and meat extract broth with and without glucose and containing the dye in various dilutions were tried. Counts were made of the number of paratyphoid bacilli inoculated and the number per loop of the broth after incubation. The glucose extract broth gave the most abundant growth. This medium was therefore used in our subsequent work. For all our work we have used media neutral to phenolphthalein.

Some experiments had shown that slight changes would lower the threshold of growth for the typhoid bacillus but make little change in the restraining action of the dye towards the coli types; for instance, small additions of proteid substances change the action of the dye. A test was made, therefore, of the influence of added feces as a preliminary to selecting dilutions for work with stools. Typhoid bacilli, being less resistant, were used for this test. The results with Bayer's brilliant green (Table IV) show that about one-third of the activity of the dye is lost.

TABLE IV

	No. per Loop after 18 Hours' Incubation		
	500,000	400,000	300,000
Typhoid (213 bacilli inoculated)...........	218	o	o
Typhoid+o.1.c.c. of boiled feces (213 typhoid bacilli inoculated).....................	+++[8]	+++	+++
o.1.c.c. of boiled feces (control)............	o	o	o

[8] Like plain broth control.

As a further index to the selection of dilutions, the possible variation of different strains to the dye and to different types of the dye was determined. Further strains were tried in broth containing Bayer's brilliant green. The number of bacilli inoculated ranged from one to seventy. No growth, as determined by plating a loopful of the media, occurred in dilutions after and including 1 to 400,000. In 1 to 500,000 two showed multiplication. A loop plated from these tubes showed only 24 and 500 colonies. Repeating this with six strains with Grübler's dye gave essentially similar results. Two strains, however, showed slight multiplication at the 1 to 400,000 dilution.

Because of these results dilutions of 1 to 500,000 to 1 to 100,000 were tried in testing the stools from typhoid carriers. The stools were from three cases and were examined on two occasions, once with Bayer's dye only, and once with both makes of dye. The broth was used in ten cubic centimeter amounts, and 0.1 of a cubic centimeter of thinned feces was added to each tube. The results are given in Table V.

That we can enrich typhoid by this method is evident. The results confirm those of Browning, Gilmour, and Mackie. We also agree with their suggestion to use graded dilutions. When we add feces we are introducing a variable factor and the typhoid strains also vary somewhat in their resistance to the dye. Success depends on the right adjustment. The value of the method must rest on the results of routine examinations over a period of time. From our results we would suggest the use of the three dilutions as given, and the addition of 0.1 of a cubic centimeter of thinned feces. The overgrowth of some of the plates suggests the advisability of streaking two plates from each tube, without burning the wire between the plates. Unsuccessful results from overgrowth are apparently due most frequently to *Bacillus aerogenes* types, which are extremely resistant to the dye and usurp the plate where seeding is too heavy.

The range of variability of the available paratyphoid-enteritidis strains was determined. Table VI gives the results.

The results show a wide range of variability. Certain members of this group, as already noted, failed completely to grow. How far such variations will be present in strains in human feces will be shown only by the results of routine examinations.

TABLE V[9]

RESULTS OF EXAMINATION OF STOOLS

Name of Case	Direct Plates (Conradi)	Conradi Plates after Enrichment in Broth Containing Brilliant Green			Source of Dye
		1: 500,000	1: 400,000	1: 300,000	
R.	Less than 1% typhoid	10% typhoid[10]	Over 50% typhoid; many bluish colonies not typhoid	Sterile (lower dilutions also sterile)	Bayer
M.	Negative (overgrowth by red colonies)	60% typhoid	75% typhoid	Overgrown by aerogenes types (lower dilutions the same)	Bayer
O.	30% typhoid	Overgrown by aerogenes types	Many hundred typhoid colonies wherever plate is free from aerogenes types	Overgrown by aerogenes types; typhoid isolated from plate (lower dilutions gave aerogenes types only)	Bayer
M.	7% typhoid	90% typhoid	95% typhoid	95% typhoid	Grübler
M.	7% typhoid	Many typhoid colonies where plate is clear of heavy red growth; plate red from diffusion	Clear parts of plate peppered with typhoid colonies; heavy red growth with diffusion of acid through plate	Moderate number of typhoid colonies; plate overgrown by aerogenes types	Bayer
O.	50% typhoid	Very many typhoid colonies where plate is free from overgrowth of aerogenes types	Few typhoid; practically overgrown by aerogenes types	Moderate number of typhoid colonies; plate overgrown by aerogenes types	Grübler
O.	50% typhoid	Very many typhoid colonies where plate is free from overgrowth of aerogenes types	Few typhoid; practically overgrown by aerogenes types	Plate overgrown by mucosus types	Bayer
R.	Five typhoid colonies or three plates	Confluent growth, red margin and bluish center, many fine bluish colonies between confluent areas; impossible to identify typhoid by direct agglutination from plate; fishings gave slow lactose-fermenting aerogenes types			Grübler
R.	Five typhoid colonies or three plates	Confluent growth, red margin and bluish center, many fine bluish colonies between confluent areas; impossible to identify typhoid by direct agglutination from plate; fishings gave slow lactose-fermenting aerogenes types			Bayer

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[9] Eight other stools were examined and were negative both by direct plating and after growth in dye broth.
[10] Verified by direct agglutinations of growth from plate or by fishing onto Russell's medium with subsequent agglutination.

The results with our cultures make us doubt the advisability of using only one dilution—1 to 6,600, as advised by Torrey. Although he grades the amount of feces, we doubt whether this would reduce the activity of the dye sufficiently to allow the growth of the less resistant types, assuming that such strains will occur in naturally infected feces. Furthemore, such low dilutions may not weed out the more resistant fecal types of bacteria any more than would the higher dilutions. Some aerogenes types are more resistant than the most resistant paratyphoid-enteritidis strains. In fact, two of Torrey's strains showed decidedly less resistance than the others, and acted more like some of our strains. His results with feces of dogs artificially infected prove the value of the method, but are no index to the correct dilutions to employ. The strains used for feeding are not stated, but were undoubtedly fully resistant to the dye. This proves nothing as to the results to be expected in routine examinations where strains may vary.

TABLE VI[11]

Culture and Type		No. Inoculated	Brilliant Green (Bayer)		No. Inoculated	Brilliant Green (Grübler)	
			No. per Loop after 18 Hours			No. per Loop after 18 Hours	
			1: 100,000	1: 10,000		1: 100,000	1: 10,000
Paratyphoid A	3	53	+ |	+	92	++	1320
	54	228	++	242	300	+ |	99
	56	364	++	++	496	++	++
	57	392	++	+ |	248	+ |	85
	58	314	++	+ |	640	++	++
	60	304	++	++	47	++	++
	62	59	++	o	112	o[12]	o
	63	234	2[12]	o	400	2[12]	o
	66	200	++	++	240	++	++
Paratyphoid, B	52	600	++	+ |	412	+ |	214
	53	282	++	++	55	++	++
	59	85	++	o	18	o[11]	o
	61	140	++	+ |	228	++	++
	65	142	++	+ |	160	++	++
Enteritidis	4	384	4[12]	o	668	552[12]	129
	47	270	++	++	160	++	+ |
	67	360	++	++	352	++	++
	70	298	++	++	480	++	+ |

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[11] + + = closely crowded colonies, like control; + | = closely crowded colonies, less than control; + = closely crowded colonies, less than preceding.

[12] Quantitatively similar restraint in 1 : 200,000.

As no naturally infected stools were at our disposal, we did not continue work in this direction. From our results with cultures, graded dilutions seem to us to be indicated in stool examinations for paratyphoid and enteritidis. Taking into consideration the fact that the feces itself will lower the activity of the dye, a series of dilutions ranging from 1 to 100,000 to 1 to 500,000 will probably cover the variations we have noted, with the exception of those strains which were completely inhibited, and restrain the fecal organisms that can be restrained.

Our results in the attempt to apply these results to a solid medium have been only partially successful. The green dyes can be used as by Conradi, but this has the disadvantage of the absence of an indicator for lactose fermentors. The use of sodium sulphite with the green dye or some other added dye, as fuchsin, has the disadvantage that the sulphite lowers the activity of the dye and a balance is obtained with difficulty. The attempt to find an indicator which would be sharp but not influence the activity of the green dye has so far been unsuccessful.

CONCLUSIONS

Several green dyes show a marked selective action for members of the typhoid-paratyphoid-colon group. This can be used for the enrichment of typhoid and paratyphoid bacilli present in feces. Forty dyes were tested with thirty strains covering all types of pathogenic bacteria. In general the dyes restrained the growth of the Gram-positive bacteria but had no effect on the growth of the Gram-negative group.

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A BACTERIOLOGICAL STUDY OF PYORRHOEA ALVEOLARIS AND APICAL ABSCESSES IN RELATION TO VACCINE THERAPY*

Charles Krumwiede, Jr., and Josephine S. Pratt

The conditions in the mouth as to temperature and available food make this cavity an ideal situation for bacterial growth. Much work has been done in the attempt to determine what constitutes the normal mouth flora and what relation this bears to the flora present in various pathological conditions. The only result so far obtained is a clear idea of the enormous complexity of the flora. Thus Miller isolated in a short time over one hundred varieties of micro-organism, and Gordon, confining his observations to streptococci alone, was able to differentiate many sub-groups. As Küster remarks, anyone attempting a systematic classification of all the bacteria that could be isolated would soon find himself exhausted. Furthermore, with our present methods, no one could hope to isolate more than a fraction of the flora present. With this complexity in mind, one cannot but be surprised at deductions that are drawn as to the etiological significance of certain types of micro-organisms in pyorrhoea.

Confining ourselves to the bacteria, including the spirochetes, we present the following data, which, in our mind, add evidence to our original contentions, which were as follows:

(1) At present, nothing is definitely known as to the relation of bacteria to pyorrhoea.

(2) The complexity of the flora is so great that we have no satisfactory basis for a rational procedure in vaccine therapy of pyorrhoea.

(3) The selection, for a vaccine, of a few types which may develop on blood-agar plates, with no idea of their relationship one to the other, must of necessity be insufficient.

(4) Stock vaccines have no *raison d'etre*.

In smears from pyorrhoeal pockets, the most striking thing is the enormous preponderance of spirochetes and fusiform bacilli. Of the former, there are found at least several morphological varieties, and these varieties may be present in the same pocket. Because of their striking preponderance, much attention has been paid to them, and much work expended on their cultivation. Noguchi has isolated several types of spirochetes, but naturally, the successful cultivation alone of these types adds no evidence as to their etiological importance. Krumwiede and Pratt isolated and studied fifteen strains of fusiform bacilli, and could find nothing distinctive, culturally, in strains from pyorrhoea. Both fusiform bacilli and spirochetes appear in many pathological conditions and can be demonstrated usually in small numbers in normal mouths. To show their variation in numbers, we examined smears from a series of persons, as follows: Healthy, clean, or nearly clean mouths, dirty mouths, gum margins irritated by crowns, and mild, incipient, moderate, or advanced pyorrhoea. Of the healthy mouths, the appearance of the smear could be predicted from inspection of the teeth. One case with unusually even, well-kept teeth showed extremely few fusiform bacilli or spirochetes. A second case, with less perfect teeth, showed a few; a third, having some tartar, showed a sharp increase in the number present. The smears from frankly dirty mouths showed many, and still more were present in smears from irritated gum margins. From this, through the various types of pyorrhoea, the numbers were so great that gradations were obscured.* In other words, spirochetes and fusiform bacilli find conditions satisfactory for multiplication with any change from the normal. We find them in caries and in various ulcerations of the mouth; what interpretation are we to make of their presence? Surely not that they are the primary etiological factor in pyorrhoea.

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* This is part of a systematic study of mouth infections which had its inception in the interest and financial aid of Dr. Arthur H. Merritt.

Many other morphological types of bacteria are found in smears from gum margins, the numbers being enormous in pyorrhoeal conditions. They comprise filamentous forms allied to the molds, bacilli of varying morphology, vibrio-types and cocci. Because of the general use in vaccine therapy of the last-mentioned group, especially the streptococci, we have studied them more fully. The term streptococcus is used in the broadest sense. All chain-producing cocci found were included; in a few of the groups, the chain formation was rudimentary or the tendency was to the formation of closely packed cocci, the few chains present apparently arising from the breaking up of these clusters. The latter types developed coherent colonies. It was thought best to study all the forms likely to be included in a vaccine as streptococci. Based on cultural characteristics, especially sugar fermentations, many types can be differentiated. This differentiation, as shown in the table, is tentative in that fermentations and action on washed blood cells are only comparative, since they were determined on one medium only (serum broth), under conditions as identical as possible. That the reactions given are final we do not believe. Comparative tests with various media have shown that some variation occurs depending on the favorability of the medium employed. Some of the inulin-fermenting types have failed to produce meth-haemaglobin which may be explained in this way. There is every reason to believe, however, that could the ideal medium be used for each strain, the resulting changes would lead to a combination of some of the groups given, but likewise to a splitting into new groups, the result being undiminished complexity.

The table was originally arranged so that the cultures to the left were the elongated types of cocci; those to the right, the flattened types, with gradations between. This arrangement is, in general, retained, but we have combined the groups with similar reactions, breaking the gradations somewhat. The simplified table seems preferable, and shows the extreme complexity of the flora sufficiently to bring out our contention. To add other morphological differences, and differences in growth on agar, in broth, and colony morphology, would lead to a greater number of groups, and less agreement between the cultures from different cases, but the added complexity would not compensate for the labor involved.

On the whole, the results warrant the deduction that many varying types of streptococci and streptococcus-like organisms are present. Not only are many groups found, but no marked agreement is evident in cultures from the different cases. Although similar types are isolated from the tonsil of one case of pyorrhoea and from a healthy mouth, the number of similar cultures encountered is not striking. This does not necessarily mean that pyorrhea has a distinctive flora, but rather that depending on the flora present in the mouth, certain types find the conditions in the pockets fitted for multiplication, and as they preponderate, they are the types isolated. This assumption finds an analogy in the observed variations in the number of fusiform bacilli and spirochetes as conditions vary from the norm.

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* The presence and number of amoebas vary in a like manner. See article by Dr. Anna W. Williams and others in this volume.

TABLE, CHARACTERISTICS OF STREPTOCOCCI

Salicin	−	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	Number of Strains	Number of Groups
Raffinose	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	+	+	−	−	−	+	+	+	+	+	−	−	−	+		
Mannite	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−	+		
Inulin	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	+	+	−	−	+	−	−	+	+	+	−	−	+	−		
Milk	C	C	−	C	C	+	+	−	−	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+		
Blood	−	−	−	−	−	−	−	−	−	−	−	−	M	M	−	−	−	M	−	M	M	M	−	M	−	M	M	−	M	M		
Pyorrhoea Case 1	1	1	..	1	..	1	7	1	4	..	1	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	19	10
Pyorrhoea Case 3	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..	1	..	2	..	6	5
Pyorrhoea Case 4	..	..	..	..	4	..	..	..	9	1	1	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	1	..	..	..	18	7
Pyorrhoea Case 46	..	2	1	..	..	2	..	..	4	..	..	..	3	..	..	2	..	..	..	1	..	4	..	1	1	..	..	..	..	..	21	10
Tonsil Case 1	..	..	..	..	..	1	3	..	..	1	..	1	1	2	6	..	..	..	..	..	..	..	1	..	..	..	..	1	1	..	18	10
Normal Saliva and Tonsil	..	..	..	..	..	..	..	..	..	..	..	..	..	7	3	..	..	1	..	..	..	..	..	..	..	2	..	2	..	..	15	5
Apical Abscess No. 14	..	..	..	..	..	1	..	..	..	1	5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	9	4
Apical Abscess No. 24	..	..	..	..	..	..	..	..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	6	1
Endocarditis Strains	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	..	1	1	..	..	..	..	1	1	3	..	1	1	11	9

Symbols: +=sugar fermented; −not fermented.
C=milk coagulated; +acid production only; −no change.
M=methhaemaglobin produced; −no action.

Further work might be done on the bacilli, vibrios, etc.; both the aerobic and anaerobic types; but what will this yield other than further complexity? On what basis are we to assume that any type isolated has any etiological importance?

On the basis of these facts, how can we justify our present methods of selecting a small, unstudied part of the total flora for vaccines? If autogenous vaccines do not represent the flora of pyorrhoea, consider the futility of stock vaccines.

The cultures obtained from a few cases of alveolar abscess and also from eleven cases of endocarditis are also classified. Their grouping is of interest not only considered separately, but also in relation to the grouping of the types isolated from pyorrhoea, tonsils and saliva.

The majority of aveolar abscesses, are, in our opinion, of haematogenous origin, and may be one of several points of localization or the only one. They may, naturally, become foci from which a later dissemination originates. In infections with this group, they are the logical source of cultures with vaccines.

When one considers the differences among the strains isolated from alveolar abscesses and endocarditis, as well as their dissimilarity from the types isolated from the mouth, the treatment of remote foci by vaccines made from a few "viridans" strains from tonsils, pyorrhoeal pockets, etc., with no further study, seems to us to rest upon an assumption of immunilogical similarity of "viridans" types. That vaccines from sources other than points of localization may influence the foci of dissemination because of group immunity reactions, cannot be denied. The little evidence we have however, indicates that cultural differences are paralleled by serological differences, although cultural and immunological identity are not necessarily associated. A systematic study is needed to determine the limitations of vaccine therapy in disseminated infections where cultures from foci of localization such as alveolar abscesses, etc., are not available. It is evident, at least, that the streptococci from suspected primary foci such as tonsils, pyorrheal pockets, etc., should be studied so that the vaccine shall contain all the types present.

The grouping of the cultures from the alveolar abscesses is interesting from another standpoint. In case 24, the conditions of extraction were ideal, and the cultures isolated fall into one and only one group. Cultures obtained from the cavity after extraction contained a second group, however, as well as diphtheroid bacilli. In Case 14, where cultures were taken from a tooth sent to us, four groups as well as bacillary types, were isolated. These observations, as well as the varied flora obtained from several other cases, where the difficulty of extracting roots made salivary contamination almost certain, seems to us of practical application. If the cultures obtained from a root fall into one, or nearly one, group according to their reactions, we may feel reasonably certain that we have the causative organism. If, however, they fall into many groups, and especially if bacillary types are present, salivary contamination has undoubtedly taken place, and the selection of one type as the causative organism may be impossible. Where sinus formation has occurred, secondary invasion from the mouth is to be expected.

SUMMARY

The data given constitute only a preliminary study of mouth conditions. The great complexity of the flora found in pyorrhoea does not suggest that vaccines made from a few of the types present would influence the pyorrhoeal condition. The cultural results show strongly the necessity of a study of the streptococcus viridans group, not only culturally, but also immunologically. Such a study should include as many strains as possible of known invasive capacity, as well as those from mucous membranes. If this is a heterogenous group, as the evidence so far obtained seems to indicate, the important bearing of such results on specific therapy is obvious. Much of the vaccine therapy as now practised rests on a doubtful assumption.

A BACTERIOLOGICAL STUDY OF AN EPIDEMIC OF SEPTIC SORE THROAT

Charles Krumwiede, Jr., and Eugenia Valentine

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The epidemic we have to report is of especial interest because of the complete and conclusive results obtained. The epidemiological facts are briefly as follows:

The outbreak occurred during June, 1914, in Rockville Centre, Long Island, a village of 4,250 inhabitants. It was soon evident that the common element among the infected persons was their milk supply. The suspected milk came from one dairy (Oceanside) housing twenty-two cows. This dairy also obtained some milk from two other small farms (R. and C.), and distributed about four hundred quarts a day to about one hundred and seventy-three customers. On investigation it was found that two hundred and five out of two hundred and thirty-two cases obtained their milk from this source. Other milkmen delivered about twelve hundred quarts a day. That is, a milk supply making up twenty-five per cent. of the total had at least ninety per cent. of the cases.

All three farms were investigated. At the Oceanside Dairy it was learned that Miss W., the daughter of the owner, developed a sore throat on April 16, 1914. On May 9th, Mrs. W., the owner, developed sore throat, but claimed that she had remained away from the dairy until May 28th. On May 11th the driver developed sore throat and on June 9th the milker. The major part of the milking was done by Mrs. W. and the milker, but the driver helped at times and possibly others. All apparently helped as needed with the work.

Isolated cases of sore throat developed in users of this milk on April 21st, 27th; May 17th and 21st. Two cases occurred in a family using milk from their own cow on May 30th. No information is available of other cases at this time, but we are inclined to believe that these as well as those on the farm were sporadic and contact cases.

From June 1st to 5th the number increased, the main part of the outbreak occurring between June 6th and 14th. A few cases a day occurred until June 30th and two isolated ones on June 29th and 30th.

The material for bacteriological investigation consisted of moist swabs from the throat of each individual on the three farms and from a number of cases, as well as a culture from a complicating peritonitis. Milk was collected separately from each quarter of the udder from all the cows.

The samples of milk on being received were placed on ice over night and smears made from the sediment. The samples from five cows showed a moderate number of streptococci in one or more quarters. One of these cows showed evidences of mastitis on physical examination. Another cow (No. 21) showed a moderate number of streptococci in samples from two quarters and an enormous number in the sample from another quarter. The milk from this quarter was flocculent, due apparently to partial coagulation of the casein and separation of the whey. All of the positive samples were inoculated on blood-agar plates and several pure cultures isolated and their characteristics determined. The samples were obtained June 15, 16, 1914.

CULTURAL CHARACTERISTICS OF STREPTOCOCCI FROM COW'S MILK.

Cultures from—	Blood Plates	Action on Washed Blood Cells.	Fermentation of *—		
			Raffinose	Mannite	Salicin
Cows 2, 3, 4, 5 and 6......	No hemolysis	No hemolysis	–	–	+
Cow 21 (3 quarters).......	Hemolysis	Hemolysis	–	–	+

*None fermented inulin.

The moist swabs from the throats of those on the farms and from the cases were received at the laboratory within about two hours after collection. The material was then streaked on the surface of blood agar plates. The varying percentage of hemolyzing streptococci present is striking. For this reason their percentage is given with the type of case.

Cases—Obtained June 15 *and* 16, 1914:

Adult—convalescent	Hemolyzing types,		50%
Child, 2 years, slight throat symptoms, marked cervical node involvement	"	"	10%
Convalescent, 15 years old............................	"	"	100%
Adult, sick one week, relapse, marked backache and depression	"	"	48%
Adult, sore throat for 36 hours, no prostration..........	"	"	20%
Adult, sore throat for three days......................	"	"	40%
Adult, sore throat, one week..........................	"	"	85%
Child, subacute stage, lymph nodes enlarged...........	"	"	10%
Child, 20 months, ill eight days, temp. 105 deg. F........	"	"	20%
Child, 6 years, convalescent..........................	"	"	20%
Child, 3 years, throat inflamed, cervical nodes enlarged..	"	"	2%
Adult, acute sore throat..............................	"	"	5%

Oceanside Dairy—Obtained June 15 *and* 16, 1914:

Mrs. W.—Sore throat, May 9th........................	Hemolyzing types,		0%
Driver—Sore throat, May 11th.........................	"	"	0.5%
Milker—Sore throat, June 9th.........................	"	"	20.0%
Washerwoman—Illness denied, enlarged tonsils.........	"	"	0.5%

Farm R.—Obtained June 15 *and* 16, 1914:

Mrs. E. R. (22)—No history of illness, enlarged tonsils.	Hemolyzing types,		30%
M. R.—No history of illness..........................	"	"	0
N. J.—No history of illness...........................	"	"	0

Farm C.—Obtained June 15 *and* 16, 1914:

Mrs. C.—No history of illness........................	Hemolyzing types,		0
H. C.—No history of illness..........................	"	"	0
M. C.—No history of illness..........................	"	"	0

Pure cultures were isolated from each case and included, not only several hemolyzing types, but also several of the non-hemolyzing types. The cultural characteristics were then determined.

On June 29th material was received from a case (Mrs. B.) which developed peritonitis as a complication. This culture was extremely important. Being of the invasive type, it was presumptively that producing the sore throat.

The non-hemolyzing types were studied only in relation to the similar ones isolated from five of the cows. Most of the strains could be excluded because of their ability to ferment inulin or raffinose, to produce meth-hemoglobin or inability to ferment salicin. Very few strains had the fermentative characteristics of the bovine strains and nearly all which did came from the throats of the non-exposed on Farms R and C. Because of these findings the non-hemolyzing types can be excluded.

The hemolyzing types obtained from the throats of the cases and of the persons on the Oceanside Dairy and the one culture from a complicating peritonitis were not only the same in their cultural characteristics, but were similar to the hemolyzing strains isolated from the one cow. (See Table.)

Only one hemolyzing type was isolated among the non-exposed (Mrs. E. R. (22), Farm R.). This strain, although it gave the same sugar fermentations, was different in many of its characteristics. Its growth in broth was very abundant, forming a thick soft flocculent sediment, which showed a faint orange pigmentation. It fermented salicin with great rapidity and gave positive fermentation results with unfavorable media when all the other strains remained negative. Morphologically, it was sharply different from the other strains.

Rabbits were immunized with the peritonitis strain (Mrs. B.) and the serum used for agglutination. Although the serum was only of moderate titer, the results are very sharp. (See Table.)

A few streptococci from other sources were studied for comparison, including several strains from Dr. Theobald Smith, as well as cultures obtained from a widespread outbreak of sore throat in Westchester County, N. Y. (See Table.)

This outbreak of septic sore throat occurred in Westchester County during March, 1915. The investigations of Prof. C. E. A. Winslow[1] led him to believe that this outbreak was partly milk-borne and partly due to contact infection. Throat swabs were obtained from ten cases[2] and plated on blood agar. A variable percentage of slightly hemolyzing colonies were present. Because of the slight hemolysis the later material was used for poured blood plates as well. Compared with the streaked plates, the poured plates showed an apparent higher percentage of hemolyzing types and more important, typical wide zones of hemolysis. Although the streaked plates are more convenient, it is evident from this that they may give confusing results.

The cultures isolated were indistinguishable culturally from those isolated in the Rockville Centre outbreak, but were different agglutinatively. (See Table.)

The bacteriological findings in the Rockville Centre epidemic, that is, the isolation of a distinct race of streptococci from the throats of the cases and from a complicating peritonitis as well as from the throats of the persons and from a cow on one farm, and the absence of this streptococcus in the throats of persons on the other farms, these facts added to the epidemiological data, that is, the presence of sore throat on the one farm before the general outbreak, give an almost complete certainty that the following sequence of events took place:

Miss W., April 16th. Sore throat; contact infection to Mrs. W., May 9th; contact infection from one of these to driver, May 11th; one of the latter two, probably Mrs. W., infected the cow; multiplication of the streptococci in the milk ducts and in the milk itself with contamination of the mixed milk, culminating in the outbreak.

The facts, therefore, give strong added evidence to the view that infection in milk-borne sore throat is of human and not of bovine origin. The fact that the cow infected with the "human" streptococci had no physical evidences of mastitis, whereas another cow having mastitis yielded another unrelated "bovine" variety of streptococcus, is also of value as evidence in this connection. Previous observations have shown that some types of human streptococci can multiply for a shorter or longer time in the milk of the udder without producing evident mastitis.

It would be needless repetition to present the existing evidence that the streptococcus of milk-borne septic sore throat is of human origin and not due to the streptococci associated with udder inflammation, some strains of which may accidentally possess a high virulence for man. Smith and Brown[3] have given a complete analysis of the evidence furnished by their own investigations and by others.

The streptococci isolated in the various epidemics including our own have all been the same culturally with one exception, viz., "Outbreak A" of Smith and Brown. This streptococcus fermented mannite as well as salicin, but did not ferment lactose. It differed also agglutinatively from the streptococci of the Baltimore and Chicago outbreaks. Our own results show, however, that streptococci similar culturally, but from different outbreaks, are not necessarily identical agglutinatively.

Smith and Brown also show that the isolation from the suspected milk of a streptococcus culturally similar to that obtained from cases of sore throat is insufficient to establish the causal relationship. They isolated hemolyzing streptococci, culturally similar, from two cows. One produced quantitatively more acid from dextrose, lactose, saccharose, and maltose. The strain from the other cow agreed quantitatively and in virulence with the human strains. These quantitative differences were associated with differences in agglutinations.

A few points of practical value were noted in our study. One is that relatively few hemolyzing types may be found even where the swabs are inoculated directly upon blood agar. It is evident that they might easily be lost if mixed cultures on Loeffler's blood serum were submitted for examination. Typical hemolysis may not be present on surface streaked blood plates, although it may develop after longer incubation. Serum water is unsuited for the determination of sugar fermentation. All of the type strains failed to ferment salicin using this medium, although prompt fermentation took place using serum broth or serum water containing one per cent. of peptone.

Summary

Streptococci similar culturally and identical in their agglutination were isolated from cases of septic sore throat and from the udder of one cow, showing no evidence of mastitis, except the peculiar character of the milk from one quarter. The bacteriological as well as the epidemiological facts almost certainly show that the infection was primarily of human origin. In tracing the source of such an epidemic, the effort should be towards finding cases of sore throat among those engaged in producing the milk, not mastitis in the cow alone. If human streptococci are found in mastitis, they are most likely secondary agents in an already existing inflammation due to bovine strains. The streptococci in different epidemics differ culturally and those similar culturally differ in their immunity reactions. Cultural similarity of strains from man and cattle is insufficient to prove their identity. Cultural identity in every detail or immunological identity is essential.

[We are indebted to Dr. Hermann M. Biggs, Commissioner of Health, State Department of Health, N. Y., for the opportunity of studying this epidemic, and to Dr. Frank Overton, County Supervisor; Dr. A. D. Jacques, Health Officer of Rockville

Centre; Dr. H. D. Gill, Veterinarian of the Department of Agriculture, as well as Dr. M. C. Schroeder and Mr. Russell Sturgis of the New York City Health Department, who coöperated in the epidemiological investigation and supplied us with much of the material for laboratory study. A report of the general results of the investigation was given in "The Monthly Bulletin," New York State Department of Health, July, 1914.]

TABLE.—HEMOLYTIC STREPTOCOCCI.

<table>
<tr><th rowspan="2"></th><th rowspan="2">Strains</th><th colspan="3">Fermentation of—</th><th colspan="4">Agglutination, Serum Strains Mrs. B.</th></tr>
<tr><th>Raffinose</th><th>Mannite</th><th>Salicin</th><th>1–10</th><th>1–50</th><th>1–100</th><th>Contro</th></tr>
<tr><td rowspan="7">Rockville Centre.</td><td>Sore throat</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>+ |</td><td>−</td></tr>
<tr><td>Sore throat</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>+ |</td><td>−</td></tr>
<tr><td>Sore throat</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>+</td><td>−</td></tr>
<tr><td>Mrs. B., peritonitis</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>+ |</td><td>−</td></tr>
<tr><td>Cow 21, a</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>+</td><td>−</td></tr>
<tr><td>Cow 21, b</td><td>−</td><td>−</td><td>+</td><td>++</td><td>++</td><td>++</td><td>−</td></tr>
<tr><td>Mrs. E. R. (22)</td><td>−</td><td>−</td><td>+</td><td>+ |</td><td>−</td><td>−</td><td>−</td></tr>
<tr><td rowspan="2">West-chester.</td><td>Sore throat</td><td>−</td><td>−</td><td>+</td><td>+ |</td><td>−</td><td>−</td><td>−</td></tr>
<tr><td>Sore throat</td><td>−</td><td>−</td><td>+</td><td>++</td><td>±</td><td>±</td><td>±</td></tr>
<tr><td rowspan="4">Miscel-laneous.</td><td>Spinal fluid</td><td>−</td><td>−</td><td>+</td><td>++</td><td>±</td><td>±</td><td>±</td></tr>
<tr><td>Mastoid abscess</td><td>−</td><td>−</td><td>+</td><td>+ |</td><td>±</td><td>±</td><td>±</td></tr>
<tr><td>Peritonitis</td><td>−</td><td>+</td><td>+</td><td>+</td><td>±</td><td>±</td><td>±</td></tr>
<tr><td>Otitis media</td><td>+</td><td>−</td><td>+</td><td>+</td><td>−</td><td>−</td><td>−</td></tr>
<tr><td rowspan="2">Smith.</td><td>Outbreak A</td><td>−</td><td>+</td><td>+</td><td>+</td><td>−</td><td>−</td><td>−</td></tr>
<tr><td>Outbreak B</td><td>−</td><td>−</td><td>+</td><td>+</td><td>−</td><td>−</td><td>−</td></tr>
</table>

REFERENCES.

1. Preliminary Report, Mount Vernon Medical Society, May, 1915.
2. Through the courtesy of Dr. Oliver Austin, Health Officer of Tuckahoe, N. Y.
3. Journ. Medi. Research, 1915, xxxi, 455.

THE TYPES OF PNEUMOCOCCI IN TUBERCULOUS SPUTUM

Harold W. Lyall

The following brief report is essentially a continuation of the work previously reported on pneumococci in the sputa of tuberculous patients at the State Hospital at Ray Brook, N. Y. It was considered worth while to apply the same technic to another series of cases under as nearly as possible the same conditions in a different institution with a view to comparing the data thus obtained.

Twenty-five cases, patients at the Municipal Sanatorium, Otisville, N. Y., were studied, exactly the same procedure being used as was followed in the Ray Brook series.[1] The types of cases selected for examination approximated closely those previously reported except that in this present series it was impossible to obtain as many cases of stage III. Only cases with definite pulmonary lesions were selected and preferably those having positive sputum. Graded according to the Turban scale, this series included five cases of stage I, nineteen cases of stage II, and only one in the third stage. As will be seen in the table, there were few definite cavity cases, and, although a history of recurrent hemoptysis was obtained in several instances, no opportunity was afforded to examine any sputa at the time of hemorrhage.

Only one case (Case 14) gave a history of a recent previous pneumonia. In this instance, a severe pneumonia in December, 1914, was followed immediately by hemorrhages and at the time of examination a Group IV pneumococcus was isolated from both the washed and unwashed specimens. This strain was not, however, highly virulent.

The following table presents briefly the findings and history of the cases:

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[1] For methods and technic see previous article, Jour. Exp. Med., 1915, XXI, 2, 146.

Case	Stage Turban	T. B. in Sputum	Description of Case	Prognosis	Pneumococci in		Remarks
					Unwashed	Washed	
1	II	Neg.	Inactive, no cavity; occasional blood-streaked sputum	Good	IV	0 2 spec.	Has marked mitral regurgitation.
2	II	Pos.	Moderately active; no cavity; no hemorrhage	Doubtful	0	0	
3	III	Pos.	Active; no cavity or hemorrhage	Bad	0	0	An organism presenting the following characteristics was isolated in both unwashed and washed specimens:—Capsule +Inulin 0, Bile 0. Mucoid sediment in broth-chains. Pseudo agglutination with Sera I and II after 24 hours.
4	II	Neg.	Inactive; slight hemorrhage Jan., 1915	Good for pulmonary condition	IV	IV	Has been operated for tuberculous kidney and gastric ulcer. Has tuberculous hip and inferior maxilla.
5	II	Pos.	Inactive; occasional blood streaks	Fair	0	0	
6	II	Pos.	Inactive; slight hemorrhage, Sept., 1914	Favorable	0	0	
7	I	Neg	Inactive; no cavity or hemorrhage	Good	III	III	
8	II	Neg.	Inactive	Good	0	0	
9	I	Pos.	Inactive	Good	0	0	An organism presenting the following characteristics was isolated in both the unwashed and washed specimens:—Capsule 0, Inulin +Bile +. Sediment in broth—long chains. Pseudo agglutination with Serum I after 24 hours.
10	II	Pos.	Moderately active; has recurrent hemoptysis	Fair	0	0	
11	II	Pos.	Inactive; slight hemorrhage, March, 1914	Good	0	0	
12	II	Pos.	Inactive; no cavity	Good	0	0	
13	II	Pos.	Slightly active; no cavity; sputum blood streaked	Doubtful	III	III	
14	II	Pos.	Slightly active; severe hemorrhage, 1914. No cavity	Doubtful	IV	IV	Pneumonia—1914; sputum examination, March 1915.

Case	Stage Turban	T. B. in Sputum	Description of Case	Prognosis	Pneumococci in		Remarks
					Unwashed	Washed	
15	I	Neg.	Inactive; no cavity	Good	0	0	Asthmatic.
16	II	Pos.	Moderately active; no hemorrhage	Bad	IV	0 2 spec	
17	II	Pos.	Inactive	Good	0	0	
18	II	Pos.	Slightly active; hemorrhage, Dec., 1914	Doubtful	IV	IV	
19	II	Pos	Active; hemorrhage, Dec., 1914	Bad	0	0	
20	I	Neg.	Inactive	Good	0	0	
21	II	Pos.	Inactive	Good	0	0	
22	II	Pos.	At present inactive; hemorrhage, Dec., 1914	Good	0	0	
23	I	Pos.	Active; hemorrhage Feb.,1915. No cavity	Uncertain	IV	0 2 spec.	
24	II	Pos.	Active; no cavity	Bad	0	0	
25	II	Pos.	Inactive; hemorrhage, Oct., 1914	Good	III	III	

In the twenty-five cases, pneumococci were isolated from the unwashed specimens of nine patients, or 36 per cent. The corresponding washed specimen was positive in only six out of the nine cases. In all cases in which both the unwashed and washed specimens were positive the same type of organism was obtained in both instances.

It will be noted that no organisms of Group I or II were isolated. The relatively high frequency of organisms tentatively recorded as belonging to Group III makes the author believe, as was pointed out in the previous article, that there is a division within this group which, although not distinguishable by morphological and cultural characteristics from the true Type III pneumococcus, might possibly be demonstrated by sufficiently fine biological reactions. This supposition is in accord with the recently reported findings of Dochez and Avery.[2]

Organisms other than pneumococci were isolated much more frequently than in the Ray Brook series. This may be accounted for, possibly, by the different season of the year at which the examinations were made, *i. e.*, summer at Ray Brook and March and April in the present series. Another contributing factor may have been a moderately severe epidemic of throat infections which was prevalent at this time. Hemolytic streptococci were isolated from the peritoneal exudates of the mice in six cases, B. influenzae in one, a Friedlander bacillus in one, and in two cases a peculiar organism intermediary between the pneumococcus and streptococcus. (See note on cases 3 and 9.)

A comparison of the percentage figures for the two series shows a close correspondence to the forty-three cases examined at Ray Brook. Pneumococci were found in 36 per cent. of the cases in the present series and in 34.9 per cent. of those at Ray Brook, giving an average of 35.3 per cent. for the combined total of sixty-eight cases.

The present series of twenty-five cases of frank tuberculosis, considered together with those previously reported, substantiates the conclusions arrived at in the previous paper—that the type of pneumococci present is usually that found in the normal mouth and that the fixed types present in the majority of cases of lobar pneumonia occur only infrequently.

The author acknowledges his appreciation of the co-operation of Dr. W. L. Rathbun, and the members of the Medical Staff of the Municipal Sanatorium. He is also indebted to Miss B. M. Bradford for valuable assistance.

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[2] Dochez & Avery. The occurrence of carriers of disease-producing types of pneumococcus. Jour. Exp. Med., 1915, XXII, 1, 105.

A STUDY OF THE GROUPING OF THE MENINGOCOCCUS STRAINS

Miriam P. Olmstead, Phoebe L. DuBois, Josephine B. Neal, Rose Schweitzer

In the Spring of 1911, the members of the meningitis department started to investigate the possible grouping of strains of the meningococcus, being led thereto by Torrey's (1) success in demonstrating the division of strains of gonococcus into groups. The desirability of some definite knowledge on this point is obvious. Not only is it of academic interest, but in the preparation of anti-meningitis serum, it is of the utmost importance that, if the strains differ in their serological reactions and may be classified thus, as many groups as possible should be represented. Also in view of the large number of strains that may be collected, it is a great saving of time and media, if the multiplication of similar strains may be avoided without lessening the therapeutic value of the serum.

Three methods at once suggested themselves—agglutination, opsonic tests, and complement fixation. The agglutination method proved unsatisfactory. Not only did the strains differ greatly, some agglutinating spontaneously, others at only very low dilutions, even with very active immune serum, but the same strains varied markedly at different times, even under conditions that were, as nearly as we could make them, exactly similar.

We tried both microscopic and macroscopic agglutination tests at icebox, room and incubator temperature, finally settling on macroscopic at incubator temperature. The cultures were grown on Thallmann agar for 24 hours and washed off with normal saline. While we spent considerable time in studying agglutination, especially in connection with our standardization tests, we did very little work in titrating strains against homologous sera as the great variability in agglutination encountered by other observers as well as ourselves, showed very conclusively the impossibility of using this method in differentiating strains from each other where relatively slight but constant differences must be depended upon to establish groups. Of thirty strains tested, eight showed no agglutination with normal serum. The others varied from 1:20 to 1:500 with normal serum, 4 showing as good agglutination with the normal as with a polyvalent immune serum in which all the strains were represented. As a rule, of course, the agglutination with the immune serum was at a much higher dilution than with the normal serum. With the immune serum, the agglutination varied from 1:20 to 1:500. The agglutinability varied markedly from day to day—strain 2, which varied rather less than the other strains, nevertheless would one day give an agglutination of 1:200 and on another 1:100 with the same serum.

The work in agglutination with the meningococcus has been done mainly in an effort to differentiate this organism from closely allied gram-negative strains, to a less degree for the purpose of standardizing anti-meningitis serum.

Goodwin and von Sholly (2) in 1906 found this method fairly satisfactory for differentiating the meningococcus from other gram-negative cocci in the naso-pharynx. Absorption tests gave even better results. They say, however, "There was a great difference in the degree of agglutinability of the cultures on different days which made it very difficult to compare results quantitatively." Kolle and Wassermann (3), Krumbein and Schatiloff (4), Columbo (5), Baecher and Hachla (6), Blumenthal (7), and Arkwright

(8) report unsatisfactory results with the agglutination tests in identifying the meningococcus. Kutscher (9), Dopter (10 and 11) and Wollstein (12) report fairly satisfactory results in the differentiation of the meningococcus from other organisms. Elser and Huntoon (13) while finding agglutination useful in many cases in identifying the meningococcus, say " The relatively large number of inagglutinable strains encountered by other observers and ourselves render this method unfit for purposes of identification and differentiation." They also comment on the great variability of the reaction. " The agglutination tests made with the same strains and the same sera on different days failed to yield uniform results although the organisms were grown on the same lot of medium and under practically identical conditions." Kutscher found more satisfactory results at 55° C. than at 37° C., Wollstein's tests were made at 55° C., Elser and Huntoon found no advantage in using a temperature of 55° C. over that of 37° C.

Opsonic tests were also unsatisfactory. Few strains of meningococcus are suitable for opsonic work, as most either show spontaneous phagocytosis or are so resistant that it is impossible to use them for this purpose. Of 34 strains tested, 25 showed very marked spontaneous phagocytosis, 6 moderate and 3 slight. When these 9 strains were tested with immune serum, they showed so much resistance to phagocytosis that they were unsuitable. Moreover, even with suitable strains the differences are not well-defined enough to permit of accurate grouping.

Work with complement fixation was begun in the summer of 1911, using filtered autolysate as antigen. While the study was not carried very far, as clinical work interrupted, enough was done to convince us that the different strains tended to arrange themselves in groups.

Literature on complement fixation contains little concerning the grouping of meningococcus strains. Citron (14) has referred to the application of complement fixation for the diagnosis of epidemic meningitis by Bruch. Citron (14), Kolle and Wassermann (3), Wassermann and Leuchs (15), Baecher and Hachla (6) and Blumenthal (7) have reported on the use of complement fixation in the standardization of therapeutic immune serum. Others have used the test as a means of differentiating the meningococcus from other gram-negative organisms. Krumbein and Schatiloff (4) found the test specific, *i. e.,* that cross-fixation did not take place with other gram-negative organisms, also that the amboceptors did not run parallel with the agglutinins. Kraus and Baecher in 1909 found meningococcus serum specific with the meningococcus antigen but that not every strain makes a suitable antigen and suggested that there is probably a multiplicity of complement binding bodies. Baecher and Hachla in 1910 repeated this suggestion of the multiplicity of complement binding bodies but made no effort to divide their strains into groups by it. In differentiating meningococcus from meningococcus-like strains they found that positive results were specific, negative results of no value. Arkwright in 1911 was unable to distinguish meningococcus from gonococcus by complement fixation. Wollstein (12) found complement fixation of value in differentiating the parameningocci from normal meningococci.

It will be seen from the above that while the complement fixation method has been used extensively in titrating serum and to a slight extent for diagnosis, except for work with the para meningococcus no attempt has been made to use it in grouping the strains of meningococcus.

The rabbits for our work were inoculated by 10 intravenous injections of live culture administered every 3rd day. The dose was increased from ¼ slant to a whole slant washed off with normal saline. The cultures for inoculation were 24 hour growths in neutral veal agar.

Antigens for Complement Fixation Tests were prepared as follows:

The stock cultures were carried on ascitic veal agar, neutral to phenolphthalein. From twenty-four hour cultures transplants were made on neutral salt-free veal agar in potato tubes. The twenty-four hour growth on these was taken up with large swabs and transplanted on neutral salt-free veal agar in large wide mouthed bottles. The twenty-four hour growth on the bottle was washed off with five to ten c. c. of sterile distilled water according to the amount of growth. The suspension was autolized at 56° C. for three hours, then passed through paper pulp and a Berkefeld filter.* The antigen was put in small bottles and sterilized at 56° C. for one half hour for three consecutive days. The antigens were made isotonic just before use by the addition of one part 8.5 per cent. saline solution to nine parts antigen.

The total volume of the test was 0.5 c. c., one tenth that of the classical Wassermann. The anti-sheep system was used, cells in a 5 per cent. suspension, from one to two units of amboceptor, and guinea pig serum for complement in a 10 per cent. dilution. The system was carefully standardized each day by an amboceptor titration. Sensitized cells were used and the readings of antigen and anti-body content titrations were made the next day, after the cells had settled.

The sera were freshly inactivated each day by heating for one half hour at 56° C.

Antigen and serum titrations were performed according to the technique described in Park and Williams': "Pathogenic Microorganisms," 1914, pp. 184-187. The anticomplementary dose of antigen has been determined by testing decreasing amounts of antigen (0.4, 0.3, 0.2, 0.1, 0.05 c. c.) in various dilutions. The antigen unit, by which is meant the smallest amount of antigen that gives complete inhibition of hemolysis with about two units of homologous immune serum, has been obtained by testing decreasing amounts of antigen (0.25, 0.2, 0.15, 0.1, 0.05, 0.025 c. c.) with 0.01 c. c. of serum. The anticomplementary property of the serum has been tested in 0.04 c. c. and 0.02 c. c. of undiluted serum. The antibody unit, by which is meant the smallest amount of serum that gives complete inhibition of hemolysis with about two units of homologous antigen, has been obtained by testing decreasing amounts of serum (0.02, 0.01, and of a 10 per cent. dilution 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01 c. c.) with about 0.1 c. c. of antigen.

The work on the differentiation of Meningococcus strains was begun with neither antigen nor serum standardized. A polyvalent antigen of all the strains was made and standardized with 0.01 c. c. of serum from a horse immunized against all the strains. The polyvalent horse serum was then titrated with one and a half units of this polyvalent antigen and from one and a half to two units of the polyvalent horse serum were used in standardizing all the monovalent antigens. Each monovalent antigen was titrated in several dilutions to obtain the antigen unit. From one and a half to two units of the monovalent antigen thus standardized were used in standardizing its homologous immune serum. Any antigen so poor that double this amount was anticomplementary was discarded and not used for serum titrations. Serum was also titrated in a sufficient number of dilutions (1 in 10 and 1 in 100) to determine the anti-body unit. From one and a half to two units of serum (diluted so that 0.01 c. c. contained that amount) were used in making the antigen cross titrations. The specificity of the antigens was tested by titrating against a known positive gonococcus serum with practically negative results.

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* The Berkefeld filters used for this work were bottled in sterile distilled water until they were neutral to phenolphthalein.

Rabbits were immunized against forty strains of Meningococcus but the cross fixation work was carried out completely with only twenty-nine of these strains. Each antigen and each serum were titrated against all the strains in several dilutions until the smallest amount of both antigen and serum that gave complete inhibition of hemolysis was determined. Table I gives the results of the antigen cross titrations and Table II the results of the anti-body content titrations.

Of the twenty-nine strains of Meningococcus tested, fourteen fell into one group, eight into another, two cross fixed with each other only by both antigen and serum titrations, two did not fix with any other strain and three acted irregularly. Strains 1, 3, 4, 7, 8, 19, 20, 21, 22, 23, 24, 26, 28, 34, cross fixed with each other, forming Group I (tables I and II). Strains 9, 10, 11, 13, 14, 16, 18, 32 cross fixed with each other, forming Group II, but antigens 9, 10, 16 of Group II fixed some sera of Group I. Antigen titrations only of strains 21 and 26 were made against other single strains, as the rabbit sera of these strains lost their anti-body content before serum titrations could be performed. These strains were not included in an antigen made of six strains (3, 21, 10, 18, 2 and 12). The sera of these strains (21 and 26), however, gave fixation with this polyvalent antigen and the antigens of these strains fixed all the sera of Group I, hence, we concluded that these strains belonged to Group I. Antigens of strains 6 and 29 gave fixation with their homologous sera only and sera 6 and 29 gave fixation with their homologous antigens only. Antigens and sera of strains 15 and 17 cross fixed with each other. Strains 2, 12 and 27 acted irregularly. In the antigen titrations of these strains cross fixation occurred with the sera of Group I, but the immune sera of strains 2, 12, and 27 fixed complement with their homologous antigens only.

Sera of twelve strains 1, 3, 4, 7, 8, 19, 20, 21, 23, 24, 26, 28 of Group I, seven strains 9, 10, 11, 13, 14, 16, 18 of Group II, the irregular strains 2, 12, 27, the odd strains 6, 29, 15, 17 and nine unidentified strains 31, 32, 33, 34, 36, 37, 38, 39, 40 were titrated with a polyvalent antigen consisting of strains 3 and 21 of Group I, 10 and 18 of Group II, and the irregular strains 2 and 12. The relationship of strains 6, 15, 17, 27 and 29 had not been determined at the time this antigen was made or they would have been included. These strains only gave no fixation with the antigen, but our later work demonstrated that they were not related to any of the strains included in the antigen.

An antigen of nine strains consisting of strains 3 and 28 of Group I, 10 and 18 of Group II, 2, 12 and 27, which acted irregularly, 6 and 29 which were not related to any other strains, was made and tested against all the sera that we knew had not lost anti-body content and also against the strains that were not included in the cross-fixation work. All sera tested with this antigen gave complete fixation.

As stated above, the monovalent antigens were standardized with the polyvalent immune horse serum for the serum cross titrations, which were made before the antigen cross titrations. We found after we had done the antigen cross titrations, that the antigens gave a much longer range of fixation with their homologous immune rabbit serum than with the polyvalent horse serum. It seems, therefore, that so much antigen was used in the serum cross titrations that a group reaction occurred. This may explain why a more distinct differentiation of strains was obtained by means of antigen titrations than by means of anti-body content titrations.

Complement Fixation is such a delicate method of differentiating strains of an organism that it is difficult to obtain absolutely consistent results. The age and anti-body content of a serum, the range of the antigen, the strength of the complement, the interval between the completion of the test and the reading of the same, the temperature of the ice-box, and the frequency of standardization of sera and antigens are factors that greatly influence the test.

Had this piece of work been performed with sera not more than three months old, results would have been more satisfactory. The differentiation by both anti-body content and antigen titrations was much more distinct with the fresh serum. As the sera became older, many of them lost their specific anti-bodies, while the reaction with the heterologous strains remained about the same, *e. g.,* the anti-body unit of immune rabbit serum of strain 14 (rabbit No. 814) one month after the rabbit was bled was 0.0005 c. c., with the heterologous antigens the unit was 0.004 c. c. Ten months after date of bleeding the anti-body unit of this serum was 0.004 c. c. with both homologous and heterologous strains, and the serum was useless for differentiation purposes. It is equally difficult to work with serum or antigen of low titre for they seem to give more non-specific reactions than those of high titre. To obtain absolutely consistent results, it is also necessary that both serum and antigen should be standardized each day before use, as serum loses anti-body content in time and both antigen and anti-body unit vary with different complements. It was impracticable to make such frequent standardizations, as we worked with so many strains. Much better results can be obtained if only ten or fifteen strains are differentiated at a time.

A constant amount of complement should be used in this test. While the standardization of the system by an amboceptor titration is satisfactory for diagnostic tests, we feel sure that in cross titration work better results can be obtained if complement of uniform strength is used.

The readings were nearly all made from eighteen to twenty-four hours after completion of the test, during which time the tests were stored in the ice-box. The reaction was the same whether the readings were made within eighteen or twenty-four hours, while a variation of the temperature of the ice-box affected the test. During hot months it was found inadvisable to do such work and when it was done readings were made immediately after completion of the test, before the cells settled. Tubes not easily read were centrifuged for three minutes.

A study of the history of the strains proves that the clinical history of the case has nothing to do with the grouping (Table III).

Strains causing severe reactions in rabbits—it being necessary to try several rabbits before one could be found that could be immunized—were: 1, 2, 7, 16, 18 and 24; 1, 7, 24 are in Group I; 16, 18 are in Group II; 2 is in Irregular Group. No relation exists between virulence and grouping. Neither is there any relation of grouping to length of time strains had been isolated. Group I contained oldest and newest strains.

The subject of the para meningococcus has received considerable attention in France for the last few years due to the work of Dopter (10 and 11), Menetrier (18) and others. This work has been done along both clinical and laboratory lines. Cases suffering from meningitis that did not respond to the ordinary anti-meningitis serum improved under the use of a para meningitis serum prepared by Dopter in 1912. Dopter in 1911 in studying the so-called para meningococcus, found that he could not differentiate these strains from normal meningococcus by using them as antigens with polyvalent horse serum, but that they could be differentiated by the serum of patients, as the sera of patients suffering from meningococcic meningitis did not fix a para meningococcic antigen, and the sera of patients suffering from para meningococcic meningitis did not fix with a meningococcic antigen.

Wollstein (12) in 1914 published a very careful study of the para meningococcus in which she arrived at similar conclusions using the sera of rabbits immunized with single strains of meningococcus and para meningococcus instead of the sera of patients. She concludes, " The para meningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation."

By the agglutination method strains 7 and 42 are classed by her as para-like strains. She did not give the results of complement fixation with these strains. In our work it was found by complement fixation that strain 7 fell in Group I and strain 42 in the irregular group allied to Group I.

As transplants of two Dopter para meningococcus strains, P. L. and P. M., were kindly given us by Dr. Wollstein, we were enabled to test them against our strains. We found antigens of P. L. to react with homologous serum only, antigens of P. M. to react with the sera of Group II.

Now if the para meningococcus is to be separated from the normal meningococcus by serological reactions and if agglutination and complement fixation yield different results with the same strains, the question is indeed a perplexing one. With the method used by us, the results yielded by the complement fixation test have been fairly uniform and specific. The tests have been repeated many times and have, on the whole, checked up very satisfactorily. By this method the strains have fallen into various groups, containing a varying number of strains. Shall we call all the strains except those in Group I, para strains, or shall we call them simply groups of normal meningococci? The question is purely academic, yet it is of some importance since the employment of the term " para " differentiates the strains more than seems justifiable. It is, perhaps, better therefore to classify them as belonging to a group coordinate with other meningococcus groups.

Wollstein further concludes:

" Because of the variations and irregularities of serum reactions existing among otherwise normal strains of meningococci, it does not seem either possible or desirable to separate the para meningococci into a strictly definite class. It appears desirable to consider them as constituting a special strain among meningococci, not, however, wholly consistent in itself." This conclusion we would heartily endorse.

Conclusions

By means of complement fixation the meningococcus may be clearly differentiated from allied organisms. A differentiation of individual meningococcus strains is possible by the use of refined technique, but the relationship of strains is so close that it is difficult to obtain absolutely clear cut and consistent results. Of the twenty-nine strains studied by us, fourteen seem to form one group and eight a second group. Three seem to be closely related to the first group, but have acted so irregularly that they cannot be classed with it, two have shown a relationship with each other only and two have shown a relationship to no other strain.

TABLE I

ANTIGENS OF TWENTY-TWO STRAINS TITRATED AGAINST SERA OF VARIOUS STRAINS

Antigen Strains	Immune Sera of Group I									Immune Sera of Group II						Immune Sera of Irregular Strains			Immune Sera of Odd Strains			
	1	3	4	7	20	22	23	24	28	10	11	13	14	16	18	2	12	27	6	29	15	17
1	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
8	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
19	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
21	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
23	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
26	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
28		+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
34		+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
9	−	+	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	±	−	−	−	−
10	−	+	−	−	−	−	±	−	+	+	+	+	+	+	+	−	−	±	−	−	±	−
16	−	+	−	−	−	−	−	−	±	+	+	+	+	+	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	+	−	−	−	−
32	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−
2	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−
27		+	+	+	−	+	−	−	+	−	−	−	−	−	−		−	+	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+

+ Denotes complete inhibition of hemolysis in as small an amount of antigen with heterologous as with homologous serum.
± Denotes complete inhibition of hemolysis but not in so small an amount of antigen with heterologous as with homologous serum.
− Denotes incomplete or no inhibition of hemolysis.

TABLE II

Immune Sera of Twenty-four Strains Titrated Against Various Antigens

Serum Strains	Antigens of Group I											Antigens of Group II						Antigens of Irregular Strains			Antigens of Odd Strains		
	1	3	4	7	8	19	20	22	23	24	28	9	10	13	14	16	18	2	12	27	6	29	15
1	+	+		+	+	±	±	+	±	±	±	–	–	–	–		±	–	±	–	–	–	–
3	+	+	+	+	±	±	+	+	+	+	+	+	+	+	+		±	+	±	+	–	–	–
4	+	+	+	+	±	–	+	+				–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	–	–	+	+	+	+		–	–		–		–	–	–	–	–	–	–
8	+	±	+		+		+	+				–	±	–	–		–	±	±	–	–		–
19	+	+	+	+	+		+					+	+		–		+	±	–		–	–	–
20		+	+	+	+	–	+	+				–	–	–	–	–	–	±	–		–		
22	+	+	+	+	±	±		+				±	±	±	±		±	±	±		–		
23	+	+				+	+					±	±	±			±				–	–	
24	+	+		+		+	+					+	+				±		+	+	–	–	
28	+	+		±								+	+				–	+			–	–	–
9	±	±	±	±	±			±				+	+	+	+			±	±		–		
10	±	+		±	±							+	+	+	+	+	+	±	±	–	±	–	
13	±	±	±	±	±							+	+	+				±	±		±		
14	±	±	±	±	±		+					+	+	+	+	+	+	±	±	+		–	–
16	±	±	±	–	–	–						+	+			+	+	–	–		–		
18	+	+		+	+		–					+	+			+	+	–	+		–		–
2	–	–	–		–	–		–				–	–		–	–	–	+	–	–	–	–	–
12	±	±	±		±							±	±		–			±	+		–	–	±
27	±	–	±	–			±					+	+			+	+	–		+	–		–
6	±	–	–	–	–	–	–	–	–	–	–	–	–	–				–	–	–	+	–	+
29	–	–	–	–			–					–	–		–		–	–	–		–	+	–
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	+
17	±	±	±	–	–	–	–	–	±	–	–	–	–	–	–		–	–	–	–	–	–	+

+ Denotes complete inhibition of hemolysis, in approximately the same amount of serum with homologous and heterologous strains.
± Denotes complete inhibition of hemolysis but not in so small amount of serum with heterologous as with homologous stains.
– Denotes incomplete or no inhibition of hemolysis.

TABLE III

History of Strains

Group I—

1. Isadore (Rockefeller)
3. Patpos (Rockefeller).
4. Rosenstein (Rockefeller).
7. Pittsburg (Rockefeller).
8. Glattmann (Rockefeller).
19. Fessenberg—Recovered.
20. Da Vito—Severe Case—Chr. Hydrocephalus—Death.
21. Fleisher—Moderate Case—Recovered.
22. New Rochelle—Sent in from New Rochelle—Case Not Seen.
23. John Carmody—Severe Case—Meningococcic Men., Cleared Up—Later Pneumococcic Men. Developed and Child Died.
24. Ercolino—Mild Case—Recovered.
26. Coffey—Severe Case—Chr. Hydrocephalus and Death.
28. Corsair—Sent from Philadelphia.
34. Brier—Mild Case—Recovered.

Group II—

9. Harris (Rockefeller).
10. Windsor (Rockefeller).
11. Messner—Severe Case—Recovered with Deafness.
13. Carbonetti (Bessie)—Severe Case—Recovered.
14. Schwartz—Severe Case—Died.
16. Vanaria—Moderately Severe Case—Recovered.
18. Pereine—Fluid Sent in from Outside.
32. Gardi—Died—Did Not Receive Anti-meningitis Serum.

Group III—

An irregular group, closely allied to Group I.

2. Gouveneur (Rockefeller).
12. Frances—Severe Case—Died—Complicated with Pneumonia.
27. Ferranti—Moderately Severe Case—Recovered.

Group IV—

A miscellaneous group, the strains of which do not cross fix with any other strains. 15 and 17 cross fix with each other.

6. Andredakis (Rockefeller).
29. Depalma—Seen Late—Recovered with Two Injections.
15. Lindhart—Severe Case—Blindness—Chr. Hydrocephalus—Death.
17. Burleigh—(?) Hospital Case.

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DOES THE BACILLUS ABORTUS (BANG) INFECT MAN?

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The frequent presence in milk of *Bacillus abortus,* the causative agent of contagious abortion of cattle and its pathogenicity for, or at least its ability to cause abortion in, many species of animals, raises the question whether it may not be infectious for man. Mohler and Traum[1] tested the serum of forty-two persons by the agglutination and complement-fixation reaction with negative results. Fifty-six adenoids and tonsils from milk-consuming children were injected into guinea-pigs. The material from one case produced nodular areas in the liver, but cultures remained sterile. The injection of tonsils from another patient produced typical lesions in guinea-pigs and *B. abortus* was isolated. More recently Larson and Sedgwick[2] presented further evidence of the possible infection of man. They found that some women giving a history of abortion gave a positive complement-fixation reaction using *B. abortus* as antigen. They also examined the blood of 425 children, and 73, or 17 per cent., gave positive reactions. These results were checked by the agglutination reaction, which gave parallel results. The positive reactions were among children having bone lesions, mostly diagnosed as tuberculosis and rickets. As they point out, inoculation of cultures into guinea-pigs produces bone lesions, which may be epiphyseal enlargements, as well as lesions of the lymphatic nodes, spleen, liver, kidney, testicle and lungs.

As noted, the *B. abortus* has been isolated only once from human tissues. As this was a tonsil, the bacillus may have come from the milk ingested and is of no value as evidence of infection. The presence of serum reactions are suggestive, but they again are not conclusive, failing the isolation of the bacillus from the lesions. That the ingestion of bacilli in large doses may be followed by the presence of antibodies in the blood has been demonstrated by several investigators. We have had similar results in feeding guinea-pigs with typhoid bacilli.

Because of these findings of Larson and Sedgwick, we have tested a large number of serums, using the agglutination reaction. This method was selected as simpler to carry out and because of the consensus of opinion that the agglutination reaction runs parallel with the complement-fixation reaction. Most of the cases have been infants or children who were inmates of a foundling asylum, and included cases of marasmus, rickets, enlarged lymph nodes, hypertrophied tonsils or adenoids, as well as about one hundred newly admitted infants from a few days to 2 months of age.

With few exceptions none of the serums from these infants had agglutinative power when tested in dilutions of 1:10 and 1:50. Two samples of serums from one child 3 years old, having bow legs and saber tibiae, undoubtedly rachitic, gave a good reaction as high as 1:100. The serums from two other children 2 1-2 and 5 years old, respectively, with no noteworthy clinical abnormalities, gave slight reactions in the 1:10 dilution. The serum from another child, 3 years old, having enlarged tonsils and adenoids, gave a complete reaction at 1:200, the highest dilution tried. Swabs were taken from the tonsils of this child and the material injected into guinea-pigs. Later the tonsils were excised and after being ground up were also injected into guinea-pigs. These pigs developed no agglutinins for *B. abortus,* and were negative on necropsy, as were also cultures made from their organs. The serums from five other children with enlarged tonsils and adenoids gave a moderate or marked reaction in a dilution of 1:10 only. The tonsils were obtained but the guinea-pig inoculations were without result.

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[1] Mohler and Traum: Bureau of Animal Industry, Circular 216, 1911.
[2] Larson, W. P., and Sedgwick, J. P.: Am. Jour. Dis. Child., 1913, vi, 326.

A case present in the obsteric ward of the above institution because of a seven months' miscarriage, but giving a negative Wassermann reaction, was investigated. The serum of the mother reacted up to 1:300; but became less active later, reacting only at 1:100 five months later. The serum of the child gave about the same reaction on one test. The foster-child of this woman was also tested with negative results. The vaginal discharge and milk from the mother and feces from the child were obtained and injected into guinea-pigs, with completely negative results.

The serums from twelve children having enlarged tonsils and adenoids were obtained at a dispensary. One gave a slight reaction at 1:10 dilution. The tonsils were ground up and injected into guinea-pigs without result. The serums from two other cases of miscarriage were also tested. In one case the only history obtained was of a seven months' miscarriage. The other patient had borne two dead children and more recently miscarried at four months. The Wassermann reaction was negative, but the patient was nephritic. Both serums gave no reaction even in low dilutions.

What interpretation one is justified in making from the above findings is doubtful. The serums from two children, one a child with enlarged tonsils, the other a case of miscarriage, gave unusually high reactions. Failing successful isolation of the *B. abortus,* such findings, as well as those reported by Larson, are only suggestive. We can at least say that no conclusive evidence has as yet been advanced that the *B. abortus* produces lesions in man.

THE ACTION OF ANTISEPTICS IN PASTEUR ANTIRABIC EMULSIONS

Daniel W. Poor

The following tests were made in the effort to obtain a suitable antiseptic to add to the emulsions of Pasteur antirabic virus in place of the 20 per cent. glycerine formerly in use. The objection to glycerine is the pain caused by the injections which in some cases is such an annoyance to the patients as to cause them to discontinue the treatment.

The conditions to be fulfilled by such an antiseptic are first, that it should be efficient in preventing the growth of bacteria that might contaminate the emulsions, and second that it should not materially affect the virulence of the emulsions even after a contact of two and one-half (2½) days.

The chemicals tested were chinosol, in various dilutions, camphor, menthol, carbolic acid, in various dilutions, and carbolic acid in combination with glycerine. The rabicidal effect of these chemicals on the virus was tested by intracranial inoculation of the virus into guinea pigs after its contact for various lengths of time with the chemicals.* The antisceptic effect was tested by adding a suspension of bacteria to the cord emulsion and antiseptics in bottles containing 5 to 6 c. c. each. These were kept for various lengths of time at room temperatures and then plated on agar. Plates were made from the bacterial suspension just before adding it to the cord emulsions so that some idea of the absolute as well as the relative value of the antiseptic was obtained.

1.

Rabicidal effect of a 1-10,000 solution of chinosol:

Equal weights of a two-day cord ground up with the chinosol solution and with salt solution (the latter to serve as control). Kept at room temperature (72 to 80 degress, for 24 hours, and tested as follows:

2-day cord (chinosol):
  Rabbit No. 1—Paralyzed in 18 days.
  Guinea Pig No. 1—Partially paralyzed in 8 days.
  Guinea Pig No. 2—Paralyzed in 10 days.

2-day cord (salt solution):
  Rabbit No. 1—Paralyzed in 8 days.
  Guinea Pig No. 1—Paralyzed in 7 days
  Guinea Pig No. 2—Paralyzed in 7 days

From above 2-day cord experiment it is evident that chinosol 1-10,000 weakened the virus.

2.

$$\text{Rabicidal effect of} \begin{cases} \text{Chinosol, 1-20,000} \\ \text{Chinosol, 1-30,000} \\ \text{Carbolic acid, } \tfrac{1}{3} \text{ of } 1\% \end{cases}$$

One-day cord emulsions, to which the antiseptics were added, kept two days at room temperature and then tested in original strength and diluted 1-6 by injecting each pig with ¼ c.c. intracerebrally.

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* The virus used was either two-day or one-day cord emulsion made up in the usual proportion of one-fifth inch of the cord to 3 c. c. of salt solution. This virus was used either in full strength or in various dilutions as indicated in the experiment. The antiseptics were added in proper proportion after the dilution.

Chinosol, 1-20,000—diluted 1-6:
No. 1—Dead in 19 days.
No. 2—Discharged one month later.

Chinosol, 1-30,000—diluted 1-6:
No. 1—Paralyzed in 6 days.
No. 2—Dead in 5 days.

Carbolic, ⅓ of 1%—diluted 1-6:
No. 1—Paralyzed in 12 days.
No. 2—Died of rabies in 21 days.

Control—diluted 1-6:
No. 1—Paralyzed in 12 days.
No. 2—Discharged one month later.

Chinosol, 1-20,000—undiluted:
No. 1—Discharged one month later.
No. 2—Paralyzed in 10 days.

Chinosol, 1-30,000—undiluted:
No. 1—(Not fully inoculated) partially paralyzed in 12 days.
No. 2—Paralyzed in 6 days.

Carbolic, ⅓ of 1%—undiluted:
No. 1—Paralyzed in 13 days.
No. 2—Dead 5 days later.

Control—undiluted:
No. 1—Paralyzed in 8 days.
No. 2—Paralyzed in 7 days.

Chinosol 1-20,000 has weakened the virus considerably.

3.

Antiseptic effect of
Glycerine, 20%
Carbolic acid, ⅓ of 1%
Chinosol, 1-20,000
Chinosol, 1-30,000
Menthol

A suspension of 5 different strains of air bacteria (two of which were spore bearers) was used. Approximately 50 bacteria added to each bottle. The cord emulsions with bacteria stood at room temperature for two days. Each bottle contained 6 c.c. of emulsión and antiseptic. In making the plates, 1 c.c. from each bottle was diluted in 25 c.c. of water and 1/10 c.c. plated. In other words, the growth from approximately 8 bacteria was diluted 250 times.

After 3 days at 37 C. degrees the result was as follows:

1. Glycerine, 20%—1 colony in 2 plates—average ½ colony.
2. Chinosol, 1-30,000—2 colonies in 2 plates—average 1 colony.
3. Chinosol, 1-20,000—1 surface colony in 2 plates—average ½ colony.
4. Carbolic, ⅓ of 1%—sterile—average 0 colony.
5. Menthol—43 colonies in 2 plates—average 21 colonies.
6. Control—1,098 colonies in 2 plates—average 549 colonies.

This means, *e. g.,* that the bacteria in 1 c.c. of the control had increased from 8 to 137,250, and in 1 c.c. of the 1-30,000 chinosol from 8 to 250.

After 5 days at 72 degrees F.:

1. Glycerine—Both plates sterile.
2. Carbolic, ⅓ of 1%—Both plates sterile.
3. Chinosol, 1-20,000—Both plates sterile.
4. Chinosol, 1-30,000—Average of 20 to a plate.
5. Menthol—Average of 21 to a plate.
6. Control—Average of 4,980 to a plate.

From the above experiment it is evident that menthol and chinosol 1-30,000 are of too weak antiseptic power to be of value. Camphor was also found to be unsatisfactory.

4.

Tests of the antiseptic power of ⅛ of 1% carbolic and 10% glycerine in combination, and also of ⅛ of 1% carbolic and 5% glycerine, showed no advantage of these combinations over ¼ of 1% carbolic acid.

5.

Antiseptic effect of carbolic acid in various strengths:

In this experiment the same four air organisms were used as in the previous experiments, to which was added staphylococcus aureus. About 60 bacteria were added to each bottle of 5 c.c. of cord emulsion. After 50 hours at room temperature, plates were grown at both room temperature and at 37 degrees:

The plates at 37 degrees:

	After 22 hours' growth.		After 4 days' growth.	
⅙ of 1% carbolic	Sterile		Sterile.	
Control	Innumerable		Innumerable.	
⅛ of 1% carbolic (A)	7 colonies	Average 5 colonies	8 colonies	Average 6 colonies.
⅛ of 1% carbolic (B)	3 colonies		5 colonies	
Control	Innumerable		Innumerable.	

After 5 days at room temperature the growth was as follows:

⅛ of 1% carbolic (A)	2 colonies	Average 4½ colonies.
⅛ of 1% carbolic (B)	7 colonies	
⅙ of 1% carbolic (A)	5 colonies	Average 3½ colonies.
⅙ of 1% carbolic (B)	2 colonies	
Controls	Innumerable.	

Five c.c. of the cord emulsion was used and 1/10 c.c. plated. Thus, in the case of the emulsion, to which ⅙ of 1% carbolic was added, while there was no increase in the bacteria growth at 37 degrees, after growing the plates at room temperature one finds an increase from approximately 12 to approximately 35 bacteria in each c.c.

6.

Rabicidal test of carbolic acid dilutions:

1-day cord emulsion kept two days at room temperature:

A. ⅛ of 1% carbolic:
    Guinea Pig No. 1—Partly paralyzed in 6 days.
    Guinea Pig No. 2—Fully paralyzed in 6 days.

B. Above diluted 1 in 6:
    Guinea Pig No. 1—Paralyzed in 6 days.
    Guinea Pig No. 2—Paralyzed in 7 days.

C. ⅙ of 1% carbolic:
    Guinea Pig No. 1—Paralyzed in 6 days.
    Guinea Pig No. 2—Paralyzed in 6 days.

D. Above diluted 1 in 6:
    Guinea Pig No. 1—Paralyzed in 6 days.
    Guinea Pig No. 2—Paralyzed in 7 days.

E. Control:
    Guinea Pig No. 1—Paralyzed in 6 days.
    Guinea Pig No. 2—Paralyzed in 6 days.

F. Control diluted 1 in 6:
    Guinea Pig No. 1—Beginning paralysis in 6 days; paralyzed in 7 days.
    Guinea Pig No. 2—Died prematurely.

In the dilutions used it is evident that neither ⅙ nor ⅛ of 1% carbolic affected the strength of the virus.

7.

Comparison of bactericidal effect of carbolic acid ⅙ of 1%, carbolic acid ⅕ of 1% and glycerine 20%:

By mistake, distilled water instead of salt solution was used in making up the cord emulsions.

Bacteria used: two air spore-bearers and staphylococcus aureus, one loop of each of the former and three loops of the latter.

The emulsion of these bacteria was made up as in previous experiments and 1/10 c.c. of the emulsion added to each bottle. The bottles were kept at room temperature for 48 hours; after 4 days' incubation in the thermostat the counts on the plates were as follows:

Control Plate No. 1	39 colonies	Average 31.
Control Plate No. 2	24 colonies	
⅙ of 1% carbolic, No. 1	2 colonies	Average 2.
⅙ of 1% carbolic, No. 2	2 colonies	
⅕ of 1% carbolic, No. 1	Sterile	Average 1.
⅕ of 1% carbolic, No. 2	2 colonies	
20% glycerine, No. 1	5 colonies	Average 6½.
20% glycerine, No. 2	8 colonies	

The small number of colonies in the control was undoubtedly due to the distilled water. In this experiment the carbolic acid showed greater antiseptic power than the glycerine.

8.

Rabicidal effect of ⅕ of 1% carbolic acid
and
⅙ of 1% carbolic acid.

One-day cord emulsion diluted 1-100 and treated with carbolic, ⅕ of 1%:

No. 1. Paralyzed in 6 days.
No. 2. Weak 6 days later. Dead 12 days later.

One-day cord emulsion diluted 1-100 and treated with carbolic, ⅙ of 1%:

No. 1. Discharged after 18 days.
No. 2. Paralyzed in 6 days.

One-day cord emulsion diluted 1-100 and treated with glycerine, 20%:

No. 1. Partially paralyzed in 6 days. Paralyzed in 7 days.
No. 2. Discharged after 18 days.

Control emulsion, diluted 1-1:

No. 1. Partially paralyzed in 6 days. Completely in 7 days.
No. 2. Partially paralyzed in 6 days. Completely in 7 days.

These emulsions, as in the preceding case, were made up in distilled water.

In this experiment the carbolic has affected the virus less than the glycerine, though both have weakened a little. The emulsions were kept two days at room temperature before being inoculated.

9.

Antiseptic effect of ⅕ of 1% carbolic acid
and
20% glycerine.

Bacterial emulsion was made up of 3 loops of staphylococcus aureus and 1 each of the spore-bearing bacilli. About 1,000 bacteria were added to each bottle containing 5 c.c. of cord emulsion. After standing two days at room temperature, plates containing 1/10 c.c. of emulsion showed the following counts, after two days at 37 degrees:

Carbolic, ⅕ of 1%, Plate No. 1	12 colonies	Average 12½.
Carbolic, ⅕ of 1%, Plate No. 2	13 colonies	
Control	Innumerable.	
20% glycerine, Plate No. 1	5 colonies	Average 6.
20% glycerine, Plate No. 2	7 colonies	

An analysis of the colonies was as follows:

Carbolic	cocci	8	About 33% cocci.
	bacilli	16	
Glycerine	cocci	4	About 40% cocci.
	bacilli	6	

Here the glycerine has had slightly the greater antiseptic strength. The bacteria in each c.c. decreased from 200 per c.c. to 125 per c.c. in the carbolic acid, and to 60 per c.c. in the glycerine.

10.

Rabicidal effect of ⅕ of 1% carbolic acid and 20% glycerine.

The bottles containing the one-day cord emulsions stood at room temperature for two days.

Cord emulsion in 1-200 dilution, containing ⅕ of 1% carbolic:
Guinea Pig No. 1—Paralyzed in 8 days.
Guinea Pig No. 2—Beginning paralysis in 6 days. Dead in 7 days.

Cord emulsion in 1-100 dilution, containing ⅕ of 1% carbolic:
Guinea Pig No. 1—Paralyzed in 7 days.
Guinea Pig No. 2—Paralyzed in 7 days.

Cord emulsion in 1-200 dilution, containing 20% glycerine:
Guinea Pig No. 1—Partially paralyzed in 8 days.
Guinea Pig No. 2—Discharged 18 days later.

Cord emulsion in 1-100 dilution, containing 20% glycerine:
Guinea Pig No. 1—Paralyzed in 7 days
Guinea Pig No. 2—Paralyzed in 7 days.

Control—1-200 dilution:
Guinea Pig No. 1—Dead in 8 days.
Guinea Pig No. 2—Paralyzed in 7 days.

Control—1-100 dilution:
Guinea Pig No. 1—Paralyzed in 7 days.
Here the carbolic has less effect on the virus than the glycerine.

11.

Rabicidal test of Carbolic acid, ⅕ of 1%, and Glycerine, 20%, after 19 hours at room temperature.

One-day cord emulsion. After standing 19 hours at room temperature, the emulsions treated with carbolic acid and glycerine were injected in the following dilutions:

Emulsion in 1-96 dilution, containing ⅕ of 1% carbolic:
Both pigs discharged 22 days later.

Emulsion in 1-48 dilution, containing ⅕ of 1% carbolic:
Guinea Pig No. 1—Paralyzed in 8 days.
Guinea Pig No. 2—Paralyzed in 11 days.

Emulsion in 1-12 dilution, containing ⅕ of 1% carbolic:
Both pigs paralyzed in 7 days.

Emulsion in 1-96 dilution, containing 20% glycerine:
Guinea Pig. No. 1—Discharged 22 days later.
Guinea Pig No. 2—Discharged 22 days later.

Emulsion in 1-48 dilution, containing 20% glycerine:
Guinea Pig No. 1—Paralyzed 14 days later.
Guinea Pig No. 2—Discharged 22 days later.

Emulsion in 1-12 dilution, containing 20% glycerine:
Guinea Pig No. 1—Paralyzed in 10 days.
Guinea Pig No. 2—Paralyzed in 7 days.

Control emulsion in 1-96 dilution:
Both pigs discharged 12 days later.

Control emulsion in 1-48 dilution:
Guinea Pig No. 1—Paralyzed in 6 days.
Guinea Pig No. 2—Paralyzed in 7 days.

Control emulsion in 1-12 dilution:
Both pigs paralyzed in 6 days.

In this experiment both the glycerine and carbolic acid appear to have affected the virus very slightly, but the carbolic less so than the glycerine.

In the foregoing experiments allowance was made in the rabicidal tests for the fact that, in the 20 per cent. glycerine emulsion, 1-5 of the emulsion was glycerine and only 4-5 virus. In the bactericidal tests, controls for the carbolic acid were made by adding an amount of the acid to each control plate equivalent to that carried over in the plates made from the emulsions treated with carbolic acid. Exactly 10 c.c. of agar was used in each plate.

GENERAL CONCLUSIONS.

1. Chinosol in dilutions of 1-10,000, 1-15,000, and 1-20,000 seemed to offer no advantages over other agents which are non-proprietary and more easily obtained. Dilutions of 1-10,000 appeared to affect the strength of the rabies virus; 1-30,000 and higher dilutions were not satisfactory from the antiseptic standpoint.

2. Camphor and menthol were of little value.

3. Carbolic acid in strength of 1-5 of 1 per cent. gave the best results. The rabicidal effect seemed to be very slight and less than that of 20 per cent. glycerine. The antiseptic effect, when kept in contact with the test bacteria (staphylococci and spore-bearing bacilli) for periods of time ranging from 19 hours to two and one-half (2½) days, appeared to be satisfactory and at least as good as that of 20 per cent. glycerine.

THE DESTRUCTIVE EFFECTS OF LIGHT AND DRYING AND OTHER LIVING-ROOM CONDITIONS UPON DIPHTHERIA BACILLI, STREPTOCOCCI AND STAPHYLOCOCCI

Daniel W. Poor and Charles P. Fitzpatrick

The work of the last decade on the destructive action of light energy upon pathogenic bacteria has been principally directed to the study of the action of the different rays and parts of light energy, luminous and non-luminous.

Lombard (I) has shown, for example, that with the ultra-violet rays of a quartz mercury lamp, the visible rays of which were entirely absorbed in a completely darkened room, the coli bacteria are killed within an hour.

The bactericidal power in light energy may very well be connected with the marvellously high number of vibrations. The ultra-violet has been 675 and 750 million vibrations per second and the X-ray 775 to 825 million vibrations per second.

The collective action of both the luminous and non-luminous rays has a greater killing power than any of the rays used separately or any part of light energy. The ultra-violet rays are the rays which kill most efficiently when isolated.

The mechanism necessary to kill would appear to require that the light rays come in direct contact with the pathogenic organism.

Wiesner (II) states that in consequence of the influence of the surrounding temperature, the bactericidal effects of light on bacteria exposed in the air prove them more resistant than those on a solid substratum which absorbs the heat; that disinfection through sunlight in living rooms occurs not at all or only to a slight extent. Sun disinfection out-of-doors, however, plays an invaluable role.

Much of the work on light has been carried on with reference to tuberculosis. Migneco (III) ascertained that tubercle bacilli in sputum when dried on a handkerchief in the sun lived 18 hours; on woolens they lived 30 hours.

Direct sunlight in a few minutes to a few hours, according to the thickness of the layer, kills tubercle bacilli. Tubercle bacilli in cultures exposed to *diffused light* in the neighborhood of the window, died in from 5 to 7 days. Robert Koch (V).

Twitchell (VI) found that tubercle bacilli in sputum lived 38 to 70 days when placed on carpet at ordinary temperatures in diffuse light.

The work on diphtheria bacilli showed that when scattered in the minutest drops and exposed to diffuse light, they were killed in 24 to 48 hours (XIII). When placed on threads and kept in the open air and in the room (indoors), they remained alive up to 86 days (XIV). In the pseduo-membranes of diphtheria patients, the bacillus has remained alive 14 weeks (Loeffler), 17 weeks (Park and Beebe) and five months (Roux and Yersin.) (VII.)

In *clear* water direct sunlight killed the diphtheria bacilli in 2 to 8 hours. If the water is discolored, as when colored yellow by organic matter, they live longer (XV).

Germano (VIII) found that diphtheria bacilli retained their virulence in dust for 20 to 40 days. Moist sand kept for 50 days showed considerable growth. That dried quickly was sterile in 50 days. In membrane (dried at room temperature) diphtheria and streptococci were alive at the end of the third month. The diphtheria bacillus withstands drying for a long time in dust, in tissues and in membrane.

Buckley (IX) recovered living diphtheria bacilli when dried on paper, after 6 days, when dried on wood after 8 days, on cotton and glass after 24 days, and on plaster after 37 days.

Germano (X) and Heim (XI) found that streptococci withstood drying for a month. Varying degrees of resistance were found, however, in different strains. Staphylococci could only be kept alive 7 days when placed on paper and other substances and exposed to the air. Buckley (XII) kept them in a dessicator on cotton under different conditions, and they remained alive for varying times up to 130 days. Staphylococci, most workers find, are killed by 3 to 5 hours exposure to sunlight (VII).

These results while somewhat contradictory are in the main of value. It was thought important to repeat the work on diphtheria and streptococci and staphylococci, especially in membranes. Many of the apparent inconsistencies are probably due to the emulsions not being of equal consistency. Moreover, in membranes and excretions, the unequal results probably depend upon destroying or inhibiting combinations of the bacteria or their toxins with antibodies fixed or free in the membranes, the presence of which we have no satisfactory means of determining beforehand. The results so far published chiefly give only the period of survival of the most resistant of the bacteria tested, without giving the rate of destruction of the total number.

While the work done with pure cultures gives more uniform results, these are not so valuable as the results obtained from a study of the bacteria as they occur in disease under living-room conditions.

The following data represent the results of some experiments made by us to obtain information on the effect of diffuse light, drying, exposure to saprophytic organisms and other "room conditions" on the number of Klebs Loeffler bacilli found in diphtheritic membranes and mucus and streptococci found in the mucus of scarlet fever patients.

While there is a fairly extensive literature on the effect of light and drying on pathogenic organisms both in pure culture and in pathological products, such as membranes, no reference has been found to the quantitative reduction by these agents of the bacteria as they occur in mucus and membranes. Diphtheria membranes from four cases, mucus from one case, and mucus from one case of scarlet fever have been studied.

Technique—The membranes were cut into pieces of approximately equal size, in three instances being weighed. They were then disposed in various ways according to the individual experiment. In some cases they were suspended by strings to allow full action of light and drying. In other instances they were laid on glass or unsterilized cloth (linen), in glass jars. The jars were provided with a vent above and were open at one end except for a covering of gauze. This allowed free passage of air and permitted admixture with other bacteria, but excluded flies. One piece of the membrane, to serve as control, was ground up immediately in salt solution and plates made. The other portions, after drying, were similarly ground up with a proportional amount of salt solution and the plates counted.

The mucus was treated as follows: Strips of unsterilized linen were soaked in the mucus and suspended or laid on glass as were the membranes. A small piece, used as control, was immediately cut off, thoroughly washed off in saline and plates made and counted. After drying, pieces of approximately the same size were cut off from time to time, similarly washed and the salt solution plated and counted.

One set of experiments was carried out by placing the membranes and mucus in diffuse daylight at a north window. In the other set they were placed in a closed closet kept partially dark.

Membrane No. 1—A piece of membrane obtained from the throat of a hospital diphtheria patient during the night, was placed in a sterile stoppered bottle in the ice box. The next day the membrane was divided into five approximately equal parts about ½ centimeter square. One portion, after being cut into small pieces with scissors, was ground up in a conical glass with a glass rod, salt solution being gradually added until an even suspension was obtained. Agar plates were made from various dilutions, ¼ c.c. being spread on each plate. The other portions of membrane were suspended in a sterile vaseline bottle and exposed to diffuse light at a north window.

After 24 hours' growth at 37 degrees it was evident that the dilutions in the control were too low, as all plates were uncountable. Another piece of membrane (after 24 hours drying) was therefore plated on agar as follows: The membrane was ground up in

(1) 2 c.c. of salt solution—called original suspension.

(2) ¼ c.c. of (1) diluted in 2 c.c. S. S.—dilution No. 1 (1 to 8), ¼ c.c. plated.

(3) 1 c.c. of (2) diluted in 5 c.c. S. S.—dilution No. 2 (1 to 40), ¼ c.c. plated.

(4) 1 c.c. of (3) diluted in 5 c.c. S. S.—dilution No. 8 (1 to 200), ¼ c.c. plated.

(5) 1 c.c. of (4) diluted in 5 c.c. S. S.—dilution No. 4 (1 to 1,000), ¼ c.c. plated.

(6) 1 c.c. of (5) diluted in 5 c.c. S. S.—dilution No. 5 (1 to 5,000), ¼ c.c. plated.

(7) 1 c.c. of (6) diluted in 5 c.c. S. S.—dilution No. 6 (1 to 25,000), ¼ c.c. plated.

After 44 hours' incubation at 37 degrees dilution No. 4 showed 111 colonies, all of which were diphtheria. Dilution No. 6 showed 18 colonies—all diphtheria. A comparison of these plates with the original uncountable control plates showed that a considerable reduction had occurred as the result of one day's drying.

After drying 6 days, *i. e.*, 5 days after the above count, dilution No. 1, after 2 days' incubation, showed 13 colonies, 9 of which were diphtheria. Dilution No. 2 showed 2 colonies. The other dilutions were sterile. As dilution No. 1 is 125 times stronger than dilution No. 4, one may assume that the bacilli have been reduced from 13,875 to 9 by 5 days' drying. In other words, 99.94 per cent. of the bacilli have been killed.

Dilution No. 1, after one day's drying, showed 25 colonies of staphylococcus aureus. After 6 days' drying, 1 colony. Five days' drying, therefore, showed a destruction of 96 per cent. of this organism.

After drying 13 days, the original emulsion grown on serum agar and incubated 40 hours showed 55 colonies of Klebs Loeffler bacilli. Dilution No. 1 showed 1 colony.

After 23 days' drying, plates of ascitic agar made from the original emulsion showed after 3 days' incubation 12 diphtheria colonies. Dilution No. 1 was sterile. As the original emulsion is 1,000 times stronger than dilution No. 4 of the control, it may be assumed that in the last two experiments the bacilli were reduced from 111,000 to 55 and 12 respectively.

Membrane No. 2—This membrane was considerably smaller than No. 1. It was divided into 4 approximately equal portions which weighed as follows:

(1) 0.050 gms.
(2) 0.044 gms.
(3) 0.040 gms.
(4) 0.043 gms.

One piece was ground up in salt solution and control agar plates made as in the case of membrane No. 1. Dilution No. 5 showed after 2 days' incubation at 37 degrees, 150 colonies, of which 12 were diphtheria. The other portions of membrane were placed on unsterilized linen and exposed to diffuse light in the jar previously described. After 8 days of drying one of the pieces was ground up and plates of serum agar made. No Klebs Loeffler bacilli were found even in the original suspension. Two Loeffler tubes also failed to show Klebs Loeffler bacilli.

After 14 days' drying ascitic agar plates were made, which in the original emulsion, after 5½ days' incubation, failed to show the presence of Klebs Loeffler bacilli.

After 23 days' drying the last piece of membrane was ground up in 5 drops of salt solution and planted in a tube of ascitic broth. After 3 days' incubation, diphtheria bacilli could not be positively identified, but ascitic agar plates made from this tube showed the presence of a few diphtheria colonies.

In the small pieces of membrane No. 2 the reduction in the number of bacilli was therefore very rapid, although even after 23 days of drying they were not entirely killed off, as shown in the last experiment.

Membrane No. 3—A large piece of membrane was divided into 5 portions weighing as follows:

(1) 0.293 gms.
(2) 0.291 gms.
(3) 0.248 gms.
(4) 0.139 gms.
(5) 0.289 gms.

As in the case of membrane No. 2 the pieces were ground up in a proportional amount of salt solution to that used in the control. One piece was ground up with sand as a control and dilutions made as follows:

(1) Membrane in 5 c.c. salt solution—"original suspension."
(2) ¼ c.c. of (1) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 1 (1 to 20).
(3) 1 c.c. of (2) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 2 (1 to 100).
(4) 1 c.c. of (3) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 3 (1 to 500).
(5) 1 c.c. of (4) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 4 (1 to 2,500).
(6) 1 c.c. of (5) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 5 (1 to 12,500).
(7) 1 c.c. of (6) in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 6 (1 to 62,500).

Plates were made on ascitic agar which after 2 days' incubation showed in dilution No. 6 five diphtheria colonies out of a total of 22 colonies. Dilution No. 5 showed 7 diphtheria colonies. The other portions of this membrane were placed in a jar under the same conditions as membrane No. 2.

After 4 days' drying in the light, one piece was ground up and dilutions inoculated on plain agar and serum agar. After 3 days' incubation the count was as follows:

	Plain Agar	*Serum Agar*
Dilution No. 1..........	1,186 K.L.B.	1,144 K.L.B.
Dilution No. 2..........	?	90 K.L.B.
Dilution No. 3..........	10 K.L.B.	6 K.L.B.

After 11 days' drying another portion of membrane was ground up with sand in the proper proportion of saline and ascitic agar plates made. The count after 3 days' incubation was as follows:

Dilution No. 1—7 diphtheria colonies.
Dilution No. 2—3 diphtheria colonies.
Dilution No. 3—sterile.
Dilution No. 4—sterile.

Loeffler tubes made from the original emulsion showed only cocci. This indicates a survival of 0.1 of 1 per cent. after 11 days' drying.

One piece of membrane No. 3 was kept on the bottom of a cotton plugged sterile bottle which was itself in a glass dish, exposed to diffuse light. In this case much of the light passed through two layers of glass and the drying process was not so rapid. After 19 days' exposure ascitic agar plates showed 130 diphtheria colonies in dilution No. 1 and 24 in dilution No. 2 after 40 hours' incubation. After 6 days' incubation the count was unchanged. Under the above conditions 3 per cent. of the bacilli remained alive.

The last piece of membrane No. 3 kept in the large jar on cloth as in the case of the first three pieces, was tested after 28 days' drying. Ascitic agar plates of all dilutions and the original emulsion failed to show K.L.B. colonies. An ascitic broth tube containing ¼ c.c. of the original emulsion showed no diphtheria bacilli after 5 days' incubation.

Membrane No. 4—This was also a large piece of membrane which was divided into four portions, giving the following weights:

(1) 0.158 gms.
(2) 0.093 gms.
(3) 0.129 gms.
(4) 0.177 gms.

The control was ground up with sand and plated on ascitic agar in the same dilutions as in the case of membrane No. 3.

After 2 days' incubation, dilution No. 4 showed 100 colonies, of which 20 were diphtheria. Dilution No. 5 also showed 20 colonies, all of which were diphtheria. The other three portions were placed in the drying jar in a closed closet, kept almost completely dark.

After 8 days, dilution No. 1 showed 444 diphtheria colonies, dilution No. 2, 41 colonies, in ascitic agar plates after 2 days' incubation. Estimating on 20 bacilli in dilution No. 4 of the control and 444 in dilution No. 1 of the dried membrane, one may suppose a survival of 17 per cent. of bacilli under these conditions.

After 23 days, ascitic agar plates showed 783 colonies in the original emulsion—all K.L.B., a reduction to 2.6 per cent. of the original.

After 64 days, the smallest piece of membrane weighing 0.093 gm. was tested out. Two ascitic agar plates of the original emulsion showed after 7 days' incubation 7 and 5 colonies respectively, none of which proved to be diphtheria. Two ascitic broth tubes inoculated with ¼ c.c. each of the original emulsion also failed to show Loeffler bacilli after 2 days' incubation.

Diphtheritic Mucus

Strips of unsterilized linen, about ½ cm. wide, were soaked in the mucus. They were then placed in the glass drying jars described above, some being suspended by strings and others laid on Petri dishes. Pieces about ½ cm. square were cut off and tested from time to time. One piece which served as control was immediately tested by washing off the mucus in 2 c.c. salt solution and making the following dilutions:

No. 1—original suspension, ¼ c.c. plated.

No. 2—¼ c.c. of No. 1 in 2 c.c. salt solution, ¼ c.c. plated—dilution No. 1 (1 to 8).

No. 3—1 c.c. of No. 2 in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 2 (1 to 40).

No. 4—1 c.c. of No. 3 in 5 c.c. salt solution, ¼ c.c. plated—dilution No. 3 (1 to 200), etc.

Plates made of plain agar.

After 2 days' incubation at 37 degrees, dilution No. 4 was estimated to contain 18 diphtheria colonies. Some difficulty was met with in obtaining an accurate count due to the prevalence of spreaders.

After 9 days' drying in the light a piece cut from a linen strip suspended in the jar, failed to show the presence of K.L.B. Cultures were made on serum agar; plain agar and ascitic agar, and were grown three days. Two Loeffler serum tubes made from the original suspension also failed to show Loeffler. The same result was obtained with the strip laid on glass.

After 15 days in the dark closet, the result in the case of the suspended strip was as follows:

Ascitic agar plates, 2 days' incubation:
  Original suspension—uncountable.
  Dilution No. 1—uncountable.
  Dilution No. 3—524 colonies K.L.B.

The strip on glass showed:
  Original suspension—uncountable.
  Dilution No. 2—601 diphtheria colonies.

The apparent increase in the number of K.L.B. on the strips after 15 days in the closet over the number in the control may be explained partly by the difficulty of obtaining an accurate count in the control due to the presence of spreaders, and also by the fact that plain agar was used in the control and ascitic agar in the later cultures.

After 16 days' drying in the light another test with suspended linen, on ascitic agar, showed:

  Original suspension—2 K.L.B. colonies.
  Dilution No. 1—0 K.L.B. colonies.

Linen on glass:
  Original suspension—1 K.L.B. colonies.
  Dilution No. 1—0 K.L.B. colonies.

Loeffler serum tube—Sterile.

After 24 days, another piece of the strip on glass was incubated for 4 days in ascitic broth and failed to show K.L.B.

Repeated in 35 days on ascitic broth culture, incubated 5 days, showed no bacilli.

After 30 days in the closet a suspended strip showed in dilution No. 1 27 colonies of K.L.B. and 22 non-diphtheritic colonies.

Loeffler tube—K.L.B.

After 39 days in the closet, suspended strip, ascitic agar, after 3 days' incubation:

Original suspension—271 colonies, 214 K.L.B.
Dilution No. 1—17 colonies, 8 K.L.B.

After 5 days' incubation:

Original suspension—285 colonies, 233 K.L.B.
Dilution No. 1—34 colonies, 14 K.L.B.

Strip on glass, after 5 days' incubation:

Original suspension—51 colonies, 0 K.L.B.

After 70 days, a strip in the closet, suspended, showed no K.L.B. in ascitic broth after one week's incubation.

MUCUS FROM SCARLET FEVER

This was treated as the other sample of mucus. The control on ascitic agar, after 3 days' incubation, showed the following count:

Dilution No. 6—34 colonies:

15, or 44 per cent.—haemolyzing streptococcus colonies.
2—non-haemolyzing streptococcus.
7—pneumococcus.
2—staphylococcus.
8—other forms of cocci.

Dilution No. 5:

30, or 54 per cent.—haemolyzing streptococci.
9, or 13 per cent.—non-haemolyzing streptococci.

After 7 days' drying in the light, both the suspended strip and those on glass failed to show the presence of streptococci after 5 days' incubation on ascitic agar plates. Total number of colonies in 4 plates (dilutions No. 1 to No. 4), suspended strip—10; on glass—15. The same repeated after 9 days with the addition of ascitic broth cultures of the original suspension also failed to show the presence of any streptococci. Total number of colonies was as follows:

On glass:

Original emulsion—2 spreading colonies.
Dilution No. 1 (a)—1 colony.
Dilution No. 1 (b)—2 colonies.
Ascitic broth—spore bearing bacilli.

Suspended:

Original emulsion (a)—6 colonies.
Original emulsion (b)—6 colonies.
Dilution No. 1—Sterile.
Ascitic broth—spore bearing bacilli.

After 9 days in the closet the count from strips on glass on ascitic agar plates, after 6 days' incubation, was as follows:

Original suspension—100 colonies.
Dilution No. 1—9 colonies.
Dilution No. 2—9 colonies.
Dilution No. 3—6 colonies.
Dilution No. 4—7 colonies.

No streptococci definitely isolated from the plates, but ascitic broth tubes made from the original suspension showed streptococci.

After 17 days in the closet, another piece showed:
Original suspension—215 colonies.
Dilution No. 1 (a)—67 colonies.
Dilution No. 1 (b)—43 colonies.

Five colonies, some of which seemed fairly characteristic of streptococci, were fished on to ascitic broth and blood agar plates. They appeared to be short chain cocci, but were non-haemolyzing and evidently not the type of coccus which constituted 44 per cent. of the control.

The uneven distribution of bacteria in diphtheritic membrane and mucus together with the difficulty of obtaining a uniform suspension of the membrane, even when the grinding is done with the aid of sand, makes it impossible to consider the figures in the foregoing protocol as anything but approximate. One can, however, obtain from them a rough idea of the reduction in the number of bacteria when exposed to light and drying in varying degrees. The results are summarized in the following tables:

MEMBRANES

	Original Number of Diph. Bacilli	After 4 Days Drying	5 Days	8 Days	11 Days	13 Days	23 Days	28 Days	64 Days
I. Medium sized pieces of membrane suspended and exposed on all sides to diffuse light.	111,000 Staphylococci 200	. . . .	72 8 Staphylococci	. . . .	. . . .	55 . .	12 . .	. . . .	. . . .
II. Small pieces of membrane, weight about 0.045 gms. placed on cloth and exposed to diffuse light	60,000	. .	. .	No colonies on plates 0	. .	No colonies on plates 0	Positive growth in ascitic broth	. .	. .
III. Large pieces of membrane, average weight of 0.248 gms. on cloth in diffuse light.	231,250	2,320	. .	. .	140	. .	. .	0	. .
IV. Large pieces of membrane, average weight of 0.14 gms. on cloth in partially darkened closet.	150,000	. .	. .	8,880	. .	. .	783	. .	0

DIPHTHERITIC MUCUS

	Original Number of Diph. Bacilli	After 9 Days	15 Days	16 Days	24 Days	30 Days	35 Days	39 Days	70 Days
Diphtheritic mucus on linen suspended in diffuse light......	18,000	0	. .	2	. .	. .	. .	. .	. .
Diphtheritic mucus on linen laid on glass and exposed to diffuse light.....................	18,000	0	. .	1	0	. .	0	. .	. .
Diphtheritic mucus on linen suspended in partially darkened closet....................	18,000	. .	104,800	. .	. .	216	. .	214	0
Diphtheritic mucus on linen laid on glass in partially darkened closet....................	18,000	. .	24,040	. .	. .	. .	. .	0	. .

	Original Number of Haemolyzing Streptococci	After 7 Days	9 Days	17 Days
Scarlatinal mucus on linen suspended in the light...........	37,500	0	0	..
Scarlatinal mucus on linen laid on glass in diffuse light...........	37,500	0	0	..
Scarlatinal mucus on linen laid on glass in partially darkened closet.........................	37,500	..	+ Streptococci found in broth culture	No haemolyzing Streptococci found

Conclusions

1. When exposed to diffuse light, drying, and admixture of dust and other bacteria, diphtheria bacilli, staphylococci and streptococci in membrane and mucus, are rapidly reduced in numbers during the first few days. After that the reduction is more gradual. This may be due to the greater resistance of certain individual bacteria or their greater protection from destruction. The streptococci seldom resist drying and light longer than a week, and the diphtheria bacilli more than three weeks.
2. Ascitic broth cultures may show the presence of a few surviving diphtheria bacilli and streptococci after the plate cultures have failed to show this.
3. Diphtheria bacilli in one membrane grew as profusely on plain agar as on serum agar or ascitic agar. In other instances the use of ascitic agar caused a more profuse growth.
4. The reduction in the number of bacteria in mucus and in membranes takes place much more slowly if the light is partially excluded as would be the case, for example, where membranes were deposited under a bed or in a dark closet. None of the streptococci remained alive as long as three weeks, and none of the diphtheria bacilli as long as ten weeks.

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TYPES OF PNEUMOCOCCI IN A SERIES OF PNEUMONIA CASES AND IN PNEUMOCOCCUS MENINGITIS

EUGENIA VALENTINE

The following observations were made to determine the incidence of pneumococci of various types in pneumonia* during the winter of 1914-15. The technique employed was that of Cole. The washed sputum was injected intraperitoneally into mice and the pneumococci in the peritoneal exudate used for agglutination. All the results were verified subsequently by the agglutination of cultures isolated from the mice. Type (Group) 3, is used tentatively only, as the identification was made on cultural characteristics.

Unfortunately, we did not receive sputa from every case available, but there was no selection of cases and the estimated death rates as given below show that this probably caused no appreciable error. The results are given in the following tables:

MORTALITY PERCENTAGES—UNSELECTED CASES OF PNEUMONIA

No data on Type.............	Recovered, 22	Died, 6 or 21%
Type determined............	Recovered, 33	Died, 8 or 19½%
Total Cases..................	Recovered, 55	Died, 14 or 20%

TYPES OF PNEUMOCOCCI IN PNEUMONIA, AND DEATH RATE ACCORDING TO TYPE (GROUP)

Type (Group)	1	2	3	4	Total
Number.............	10 or 24%	†17 or 42%	4 or 10%	10 or 24%	41
Died.................	0	6 or 35%	1 or 25%	1 or 10%	8 or 19.5%

† Two strains as well as the Type 2 strain in case 3 below, gave slow but distinct agglutination.

Other Cases Not Tabulated.

1—Influenza and Streptococcus viridans. Recovered.
2—B. Friedlander and Streptococcus Haemolyticus. Recovered.
3—Pneumonia, unresolved, Type 3 and Type 2. Died.
4—Pneumonia complicating Whooping Cough, Type 2. Recovered.

The series of pneumonia cases is noteworthy because of the high incidence of Type (Group) 2 infection as well as the low mortality in the series examined.

Pneumococcus Meningitis.

As far as we know there are very few observations on the types of pneumococci found in meningitis. A few cases have been examined with the following results:

Type 1	Type 2	Type (Group) 3	Type (Group) 4	Total
2 cases	7 cases	2 cases	2 cases	13 cases

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* We are indebted to Drs. William R. Williams and Lewis Conner of N. Y. Hospital for the sputa and to Dr. Rufus Cole of the Rockefeller Hospital for the agglutinating sera used in the tests.

The histories on these cases were as follows:

Type 1 Infections:

Case 1—No history obtainable.
Case 2—Following "Grippe," no history of pneumonia.

Type 2 Infections:

Case 1—Secondary to pneumonia.
Case 2—Secondary to pneumonia.
Case 3—"Dullness," no frank pneumonia.
Case 4—Two weeks after fracture of the skull, no evidence of pneumonia.
Case 5—One week after injury to head, fracture (?), no evidence of pneumonia.
Case 6—Chronic otitis media, no pneumonia.
Case 7—No history or evidence of pneumonia.

Type (Group) 3 Infections:

Case 1—Secondary to pneumonia.
Case 2—No history obtainable.

Group (Type) 4 Infections:

Case 1—Secondary to otitis media.
Case 2—No history obtainable.

THE PROTEINS AND ANTITOXIN IN THE SERUM OF GOATS IMMUNIZED AGAINST DIPHTHERIA

Edwin J. Banzhaf and Lemuel W. Famulener

Until protein chemistry is much further advanced, the differentiation of the several protein constitutents regarded as bodies possessing not only definite physical and chemical properties, but what may be termed functional affinities, can only be identified by salting out methods. When the value and limitation of these are understood, the isolation and association of the various antibodies with certain protein constituents for a given species will be fixed and found to be constant.

Practically all the work in the separation of the proteins with the object of identifying the various antibodies with certain proteins has been done with ammonium sulphate. Considerable confusion, however, exists in comprehending the methods and basic principles of fractioning the proteins with this salt.

With the ammonium sulphate separation of the globulins from the albumin the precipitation boundaries of these two proteins are far enough removed to allow a complete and well defined separation, thus—saturated ammonium sulphate solution is added to the serum up to 50 per cent., i. e., to 50 cubic centimeters of serum is added 50 cubic centimeters saturated ammonium sulphate solution. The separation is sharp and well defined, for there is no overlapping and pulling down of the albumin. After filtering, one may add to the clear filtrate saturated ammonium sulphate solution up to 60 per cent. without any further precipitate forming. The albumin remains in solution until the filtrate is brought up to 64 per cent. saturated solution. This holds true whether the serum contains 5 or 10 per cent. total proteins. Separation of the globulins from the albumin with this salt is easily and completely obtained. Because of this complete separation no confusion exists in the reported presence or absence of certain antibodies in the albumin.

The ammonium sulphate separation of the euglobulin from the pseudoglobulin is an artificial one, and should be abandoned. There is no dividing line, i. e., no line where the addition of a slight excess of ammonium sulphate will not cause further precipitation as there exists between the globulins and albumin. In practice investigators have commonly used the fractional precipitation with ammonium sulphate solution to separate these proteins. Thus a half volume of saturated ammonium sulphate solution is added to the serum (66 2/3 cubic centimeters of serum and 33 1/3 cubic centimeters of saturated ammonium sulphate solution). The precipitate is considered euglobulin. To purify this protein it is taken up with water and reprecipitated with ammonium sulphate several times. This so-called euglobulin obtained in this manner from an immunized horse, may or may not contain antitoxin and pseudoglobulin. This will depend on the amount of protein in the serum.

We have attempted to discover a line of division for the separation of these two proteins by using various dilutions of known protein content with various amounts of ammonium sulphate. At no point were we able to separate the two globulins, i. e., according to the separation of the globulins by saturating with sodium chloride.

The sodium chloride gives a sharp and well defined separation of the euglobulin and pseudoglobulin. Freund and Joachim[1] were the first to point out that all the euglobulin was precipitated by saturating with sodium chloride, whereas the pseudoglobulin remained in solution. One of us with Gibson[2] urged the use of this salt for the separation.

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[1] Freund and Joachim Zeit. f. phsiol. chem., 1902, No. XXXVI, 407.

When this salt is used for the separation of the globulin from horses immunized against diphtheria or tetanus toxin and the euglobulin freed from the pseudoglobulin by repeated precipitation, no antitoxin will be found in the euglobulin. We have never found antitoxin in the euglobulin of horses separated in this manner. We found that this holds true with all horses immunized against diphtheria or tetanus toxin, whether refractory or not, whether the horse is on the rise or fall of antitoxin accumulation, and whether the horse has had one or a hundred bleedings. Also in those instances when, after prolonged immunization and frequent bleedings, the horse's serum has, as sometimes happens, returned to its normal protein content and normal distribution of protein, as well as where the injections of the respective toxins have ceased for a month or a year, the antitoxin present will always be found with the pseudoglobulin, i. e., that globulin soluble in saturated sodium chloride and none in the euglobulin.

We do not mean to convey that antitoxin is actually pseudoglobulin or that the activity of the antitoxin depends on its being a pseudoglobulin or, if a chemical or physical combination does exist between the two, that this combination is essential for the activity of the antitoxin. One of us[3] was the first to show that the antitoxin can be dissociated from the pseudoglobulin without losing its identity. Formerly, all the evidence was in favor of the pseudoglobulin nature of the antitoxin. The antitoxin had every known characteristic of this protein. Now, however, we know that we can heat the pseudoglobulin containing the antitoxin and actually change the precipitation characteristics of about one-third of it, i. e., from the pseudoglobulin to the euglobulin precipitation characteristics without loss of antitoxin. All the antitoxin then in the serum of the horse is associated only with the pseudoglobulin.

In the serum from the goat immunized against diphtheria toxin, Pick[4] states that the euglobulin carries the antitoxin. Ledingham[5] reports that during the course of immunization the antitoxin is sometimes found in the pseudoglobulin and sometimes in the euglobulin. Gibson and Collins[6] found that about one-seventh of the antitoxin was in the euglobulin. These investigators used ammonium sulphate as the separating salt for the euglobulin and pseudoglobulin.

If, as Ledingham states, the antitoxin in goat serum does fluctuate in its association with the pseudoglobulin and euglobulin, then there is in the goat a changeable metabolic activity that can alter the antitoxin molecules so as to associate themselves either with the pseudoglobulin or with the euglobulin and still retain its specific neutralizing power of diphtheria toxin.

In order to determine this phase of association and relationship, we immunized these goats against diphtheria toxin. These goats were bled at various intervals, usually 100 cubic centimeters. The proteins were separated and tested for potency, and the protein content determined by heat coagulation.

The separation of the proteins for potency tests was carried out by diluting the goat serum with an equal volume of water and then adding an equal volume of saturated ammonium sulphate solution. The precipitate

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[2] Banzhaf and Gibson. Jour. Exp. Med., vol. XII, No. 3, 1910.
[3] Banzhaf, Proc. of the Soc. for Exp. Biol. and Med., 1908, VI, 24.
[4] Pick, Beitr. z. chem. Physiol. u. Path., I. p. 351, 1901.
[5] Ledingham, Jour. of Hgy., VII, 65. 1907.
[6] Gibson and Collins, Jour. Biol. Chem., vol. III, No. 4, 1907.

(pseudoglobulin and euglobulin) was dissolved in water and reprecipitated with ammonium sulphate. The precipitate was allowed to drain, dissolved in water again and then saturated with sodium chloride. The pseudoglobulin remains in solution while the euglobulin is thrown out. After filtering, the euglobulin precipitate was dissolved in water and again saturated with sodium chloride. This procedure of precipitating and dissolving was always carried out three times.

A progressive increase of total proteins was observed in the three goats, Goat No. 34 reaching the maximum six weeks after the beginning of immunization. The increase was observed in the pseudoglobulin and euglobulin. At this time the potency was twenty-five units per c.c. The maximum of Goat No. 29 was reached in about seven weeks and was eleven units per c.c. In Goat No. 28, the maximum was not reached until after three months of immunization. The potency at this time was forty units per c.c.

We also found that the unit content and protein increase are not parallel and have no relation.

Goat No. 34, after three and a half months immunization, had a potency of 100 units per c.c. The pseudoglobulin at the time had dropped below the normal, the euglobulin showing a gradual decrease to normal. Goat No. 29, after about five months immunization, had a potency of 110 units per c.c. The pseudoglobulin had dropped slightly below normal, the euglobulin showing a gradual decrease to normal. Goat No. 28, after about four and a half months immunization, had a potency of 100 units per c.c., with the protein practically in the same amount and distribution as the normal. At intervals of about a month during the course of immunization, we separated the proteins and tested them for this potency with the view of obtaining the number of units per gram protein. The accompanying tables show the distribution of the units.

At no time, as the tables show, was there an absence of units from either the pseudoglobulin or euglobulin, nor was there any wide fluctuation as Ledingham found. The unit relationship per gram protein of the pseudoglobulin and euglobulin remained practically the same during the course of immunization.

TABLE I

GOAT NO. 34

No. 34 Date of Bleeding	Total Protein	Albumen	Pseudoglob.	Euglob.	Units
Normal					
1/5	6.80	3.41	2.18	1.21	...
1/14	6.97	3.60	2.15	1.22	...
2/2	7.50	2.88	3.08	1.54	2
2/10	8.17	3.02	3.40	1.65	8
2/16	8.29	2.90	3.27	2.12	25
2/22	7.77	2.79	2.82	2.16	32
2/28	7.66	2.74	2.55	2.37	65
3/6	7.53	2.54	2.80	2.19	70
3/21	7.69	2.99	2.64	2.06	90
4/14	7.48	3.53	1.91	2.04	92
4/19	7.29	3.58	1.88	1.83	100

PROTEINS SEPARATED, TESTED FOR ANTITOXIN CONTENT AND CALCULATED TO UNITS PER GRAM PROTEIN

No. 34 Date of Bleeding	Albumen Units Per Gram.	Pseudoglobulin Units Per Gram.	Euglobulin Units Per Gram.
2/16	None	3.67	5.66
3/21	None	11.36	29.12
4/19	None	17.01	35.48

TABLE II

GOAT NO. 29

No. 29 Date of Bleeding	Total Protein	Albumen	Pseudoglob.	Euglob.	Units
Normal					
2/25	7.00	3.55	2.13	1.32	...
3/3	7.10	3.60	2.15	1.35	...
3/27	7.47	3.66	2.39	1.42	2
4/14	7.87	3.90	2.37	1.60	8
4/19	7.96	3.74	2.44	1.78	11
5/14	7.60	3.66	2.14	1.80	28
5/20	7.58	3.65	2.11	1.82	35
5/26	7.64	3.65	2.20	1.79	40
7/19	7.31	3.67	2.04	1.60	110

PROTEINS SEPARATED, TESTED FOR ANTITOXIN CONTENT AND CALCULATED TO UNITS PER GRAM PROTEIN

No. 29 Date of Bleeding	Albumen Units Per Gram.	Pseudoglobulin Units Per Gram.	Euglobulin Units Per Gram.
4/19	None	2.04	3.37
5/26	None	6.81	13.96
7/19	None	18.18	38.70

TABLE III

GOAT NO. 28

No. 28 Date of Bleeding	Total Protein	Albumen	Pseudoglob.	Euglob.	Units
Normal					
2/25	6.75	3.55	1.80	1.40	....
3/3	6.84	3.88	1.74	1.22	....
3/27	6.79	3.28	2.22	1.29	...
4/5	6.89	3.13	2.09	1.67	4
4/14	6.88	3.37	1.96	1.55	10
5/2	7.02	3.78	1.88	1.36	18
5/14	7.16	3.71	1.78	1.67	30
5/20	7.52	3.68	2.06	1.78	35
5/26	7.55	3.67	2.07	1.81	40
7/2	6.55	3.36	1.88	1.31	100

PROTEINS SEPARATED, TESTED FOR ANTITOXIN CONTENT AND CALCULATED TO UNITS PER GRAM PROTEIN

No. 28 Date of Bleeding	Albumen Units Per Gram	Pseudoglobulin Units Per Gram	Euglobulin Units Per Gram
5/2	None	4.25	7.35
5/26	None	7.24	13.81
7/2	None	17.75	45.08

STUDIES ON ANTI BODIES I

ANALYSES AND NITROGEN DISTRIBUTION OF A NUMBER OF ANTISERA

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The antisera analyzed were the following:

Antidiphtheria globulin—2,900 units per cubic centimeter. Designated *A* in the tables.

Antidiphtheria globulin—975 units per cubic centimeter. Designated *H*.

Antitetanus globulin—1,900 units per cubic centimeter. Designated *B*.

Antitetanus serum—500 units per cubic centimeter. Designated *F*.

Antitetanus serum—60 units per cubic centimeter. Designated *G*.

Antistreptococcus serum. Designated *C*.

Antigonococcus serum. Designated *I*.

Antimeningococcus serum. Designated *E*.

Normal serum. Designated *D*.

The method of separating and concentrating the diphtheria and tetanus antitoxin together with the pseudoglobulin with which they are associated was carried out as follows:

The plasma from the immunized horse was diluted with an equal volume of water and heated to 61° Centigrade. An equal volume of saturated solution of ammonium sulphate was then added. The resulting precipitate contained the fibrinogen, euglobulin, pseudoglobulin and antitoxin. This precipitate was taken up with water and reprecipitated with an equal volume of saturated solution ammonium sulphate to free it from traces of albumin. After draining the precipitate, it was taken up in water. In order to separate the fibrinogen and euglobulin from the pseudoglobulin and antitoxin, the mixture was saturated with sodium chloride. The resulting precipitate filtered out. To the clear filtrate containing only pseudoglobulin and antitoxin, acetic acid was added to excess. The precipitated pseudoglobulin and antitoxin was recovered on hardened filter paper, pressed and dialyzed.

Antidiphtheria globulin designated in the tables *"A"* was prepared from the bleedings of one horse. Antidiphtheria globulin *"H"* was prepared from bleedings from nine horses.

The antitetanus globulin *"B"* was prepared from the bleedings from two horses.

The antitetanus serum *"G"* was taken from a horse that had been bled nineteen times.

The antitetanus serum *"F"* was taken from a horse that had been bled six times.

The antistreptococcus serum *"C"* represents the sixty-sixth bleeding from a horse.

The antigonococcus serum *"I"* represents the fourth bleeding from a horse.

The antimeningococcus serum *"E"* represents the twelfth bleeding from a horse.

The normal serum *"D"* represents the first bleeding from a horse.

The various antisera were placed in shallow pans and dried in a vacuum at 35° Centigrade, scaled from the pans and kept in a vacuum desicator over calcium chloride.

The hydrogen ion concentrations of these substances in aqueous solution were determined with 0.1 gram in 15 c.c. water by comparison of the color changes with various indicators with the standard solutions suggested by A. A. Noyes.[1] The results are given in table I.

TABLE I

Hydrogen Ion Concentrations of Preparations in Water

Substance	Appearance of Mixture	Indicators	Hydrogen Ion Concentrations; H.
A	Partly dissolved	Rosolic acid Cochineal	−5.5
B	Almost all dissolved	Rosolic acid	−7.0
C	Almost all dissolved	Rosolic acid Phenolphthalein	−8.5
D	Clear solution	Phenolphthalein Thymolphthalein	−9.5
E	Partly dissolved	Phenolphthalein	−8.5
F	Partly dissolved	Phenolphthalein Thymolphthalein	−9.5
G	Partly dissolved	Phenolphthalein	−8.5
H	Partly dissolved	Rosolic acid	−6.5
I	Clear solution	Phenolphthalein Thymolphthalein	−9.8

The methods of analysis were in general those commonly employed. They will be described briefly:

Moisture. The substance in a beaker or platinum dish was dried in an air bath at 100-105° for three to four hours and the loss in weight determined. The heating was repeated for one-hour periods until the weight had become constant. After heating, the substance was allowed to cool each time in a desiccator over calcium chloride.

Ash. The substance was carefully ignited in a platinum dish, finally heated with a Meeker burner after the addition of 3-4 drops of concentrated nitric acid. The heating was repeated until there was no further loss in weight. The color of the ash was white in every case.

P_2O_5. The ash was dissolved in about 25 c.c. water and a little nitric acid with warming. To the solution 12 c.c. ammonium nitrate solution (750g NH_4NO_3 dissolved in water and diluted to one liter) and 50 c.c. molybdate solution (60g MoO_3, 440 c.c. H_2O, 60 c.c. NH_4OH, sp. gr. 0.90, poured into a mixture of 250 c.c. H_2O and 250 c.c. HNO_3 sp. gr. 1.40, filtered after standing several days) were added, the mixture heated at 80° on a water bath for ten minutes, cooled and allowed to stand overnight. It was then filtered on asbestos in a Gooch crucible, washed 5 to 6 times with an NH_4NO_3 solution (containing 50g of the salt and 10 c.c. conc. HNO_3 in 1 liter) once with 1% HNO_3 solution, dried in an air oven at 120° and weighed as $(NH_4)_3PO_4 \, 12 \, MoO_3$.

Nitrogen. The Kjeldahl method with sulfuric acid, potassium sulfate, and a small amount of copper sulphate was used. The ammonia was distilled into standard sulfuric acid which was then titrated with sodium hydroxide solution with alizarin sulfonic acid as indicator. The substance was dried in an air bath at 101-104° for four hours in every case.

Table II shows the experimental data obtained in the analyses. The headings of the columns are perhaps sufficient to indicate the meaning of figures.

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[1] Jour. Amer. Chem. Soc., 32, 823 (1910).

TABLE II

Analyses of Antitoxin Preparations—Experimental Data

		Moisture		Ash		P_2O_5			Nitrogen			
Substance	Grams Taken	Loss Grams	Loss Percent.	Wt. Ash Grams	Ash Percent.	$(NH_4)_3 PO_4 12MoO_3$ Wt. Grams	Wt. P_2O_5 Grams	Percent. P_2O_5	Grams Taken	cc. o.1 N H_2SO_4	Nitrogen Grams	Nitrogen Percent.
A	0.5093	......	....	0.0033	0.65	0.0172	0.00065	0.13	0.1543	15.13	0.02120	13.74
	0.5047	......	....	0.0031	0.61	0.0164	0.00062	0.12	0.1540	15.17	0.02125	13.81
	0.5062	0.0171	3.38	......	....	......	....	....	....	.....	....	.....
B	0.5028	......	....	0.0039	0.78	0.0185	0.00070	0.14	0.1516	15.13	0.02120	13.98
	0.5049	......	....	0.0041	0.81	0.0185	0.00070	0.14	0.1525	15.41	0.02159	14.16
	0.5026	0.0148	2.94	......	....	......	....	....	......	.....	....	.....
C	0.5030	......	....	0.0097	1.93	0.0296	0.00112	0.22	0.1513	14.73	0.02064	13.64
	0.5063	......	....	0.0100	1.97	0.0294	0.00111	0.22	0.1552	15.30	0.02144	13.81
	0.5019	0.0179	3.57	......	....	......	....	....	....	.....	....	.....
D	0.5057	......	....	0.0106	2.10	0.0432	0.00163	0.32	0.1529	14.26	0.01998	13.07
	0.5056	......	....	0.0123	2.43	0.0441	0.00167	0.33	0.1531	14.34	0.02009	13.12
	0.5035	0.0167	3.32	......	....	......	....	....	......	.....	....	.....
E	0.5044	......	....	0.0084	1.67	0.0293	0.00111	0.22	0.1527	14.58	0.02043	13.38
	0.5055	......	....	0.0101	2.00	0.0298	0.00113	0.22	0.1530	14.65	0.02052	13.40
	0.5047	0.0183	3.63	......	....	......	....	....	......	.....	....	.....
F	0.5032	......	....	0.0079	1.52	0.0214	0.00081	0.16	0.1515	15.03	0.02106	13.90
	0.5016	......	....	0.0078	1.56	0.0201	0.00076	0.15	0.1507	15.04	0.02107	13.98
	0.5037	0.0241	4.78	......	....	......	....	....	......	.....	....	.....
G	0.5038	......	....	0.0099	1.97	0.0370	0.00140	0.28	0.1523	14.55	0.02038	13.38
	0.5047	......	....	0.0099	1.96	0.0360	0.00136	0.27	0.1544	14.78	0.02071	13.42
	0.5023	0.0241	4.80	......	....	......	....	....	......	.....	....	.....
H	0.5077	......	....	0.0046	0.91	0.0143	0.00054	0.11	0.1552	15.29	0.02142	13.80
	0.5017	......	....	0.0041	0.82	0.0138	0.00052	0.10	0.1527	15.01	0.02103	13.77
	0.5010	0.0264	5.27	......	....	......	....	....	......	.....	....	.....
I	0.5080	......	....	0.0113	2.22	0.0343	0.00130	0.26	0.1569	14.93	0.02092	13.33
	0.5055	......	....	0.0109	2.16	0.0344	0.00130	0.26	0.1550	14.82	0.02076	13.39
	0.5012	0.0280	5.58	......	....	......	......	....	......	.....	....	.....

The mean results of the analyses are given in Table III.

TABLE III

MEAN RESULTS OF ANALYSES OF ANTITOXIN PREPARATIONS

Substance	Per Cent. Moisture	Per Cent. Ash	Per Cent. P_2O_5	Per Cent. (Dried Material) Nitrogen
A	3.44	0.63	0.13	13.78
B	2.94	0.80	0.14	14.07
C	3.57	1.95	0.22	13.73
D	3.32	2.27	0.33	13.10
E	3.63	1.84	0.22	13.39
F	4.78	1.54	0.16	13.94
G	4.80	1.97	0.28	13.40
H	5.27	0.87	0.11	13.79
I	5.58	2.19	0.26	13.36

Since the amount of moisture adhering to the solid preparations after grinding to a fine powder and drying in a vacuum desiccator over calcium chloride appears to be variable, it appears best to compare the composition of the preparations after correcting for the moisture content.[1] Table IV shows the percentage of nitrogen and of ash of the dried preparations in columns 2 and 3; of nitrogen in the dried and ash free preparations in column 4; and finally in column 5 are given the percentages of phosphorus pentoxide in the ash of the different preparations.

TABLE IV

CORRECTED RESULTS OF ANALYSES OF PREPARATIONS

Substance	Dried Preparations		Dried and Ash Free	Per Cent. P_2O_5
	Per Cent. Nitrogen	Per Cent. Ash	Per Cent. Nitrogen	of Ash
A	13.78	0.65	13.87	20.6
B	14.07	0.82	14.19	17.5
C	13.73	2.02	14.01	11.3
D	13.10	2.35	13.42	14.5
E	13.39	1.91	13.65	12.0
F	13.94	1.62	14.17	10.4
G	13.40	2.07	13.68	14.2
H	13.79	0.92	13.92	12.6
I	13.36	2.32	13.68	11.9

In studying the results given in Table IV some interesting facts are apparent.

(1) The low percentage of ash in the moisture free globulins (A, B, H) is accounted for in the prolonged dialization of these globulins to free them from ammonium sulphate. After removal of ammonium sulphate 0.6%

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[1] The fact that drying in vacuum desiccator over calcium chloride does not remove all the moisture was shown by Falk and Sugiura (Jour. Amer. Chem. Soc. 37, 219 (1915) for enzyme preparations analogous in composition. These results show that preparations dried as stated will lose moisture when placed in a vacuum desiccator over phosphorus pentoxide or if heated to 100-110°. The loss by the last two methods was practically the same.

to 0.8% of sodium chloride is added to bring the globulins up to a near physiological sodium chloride content. For the two tetanus sera (F and G), the more active preparation (F) contained 1.62% ash, the less active 2.02% ash. The normal serum contained the highest percentage of ash.

(2) For the nitrogen content of the moisture free preparations, the less active tetanus serum (G) contained less (13.40%) than the more active (F, 13.94%). The less active diphtheria globulin (H) contained the same percentage of nitrogen as the more active (A). The nitrogen contents for all of the preparations are considerably smaller than those recorded in the literature for the proteins of horse serum.

(3) The $P_2 O_5$ contents of the ash ranged from 10% to 20%. The markedly higher values for the tetanus and more active diphtheria globulins are noteworthy. As for the rest, no very distinct differences appear except possibly the comparatively large value for the normal serum and the smaller value for the more active tetanus serum 10.4% as compared to 14.2% for the less active.

The different preparations were finely ground and extracted with dried ether in Soxhlet extractors for several days. The ether extracts on evaporation gave residues amounting to approximately 0.1 per cent. of the material extracted. The analyses of the extracted substances showed no marked differences in comparison to those of the unextracted substances.

A number of routine tests were performed with the preparations. Positive reactions were obtained for all with the Millon test for proteins,[1] the glyoxylic acid test for tryptophane,[2] the Folin test for tyrosine,[3] and the Molisch test for carbohydrates.[4]

TABLE V

FORMAL TITRATIONS OF ANTITOXIN PREPARATIONS

Substance	Titration cc 0.1 Na OH	mg N as N H_2:COOH	Per Cent. N as NH_2:COOH
A....................	0.40	0.56	0.56
B....................	0.26	0.36	0.36
C....................	0.35	0.49	0.49
D....................	0.31	0.43	0.43
E....................	0.25	0.35	0.35
F....................	0.29	0.41	0.41
G....................	0.44	0.62	0.62
H....................	0.59	0.83	0.83
I....................	0.64	0.90	0.90

At present there is no apparent regularity to be observed in these results.

Since these antitoxin preparations appeared to be essentially protein in character, an attempt was made to characterize more particularly the different groups of amino acids which were present. The methods of analysis described recently by van Slyke were used.[5] A very concise summary of the principles underlying the separation is given by Percival Hartley[6] from whom the following description is quoted.

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[1] Cf. H. C. Sherman, Methods of Organic Analysis (1912), p. 315.

[2] *Ibid.*, p. 315; also S. R. Benedict, J. Biol. Chem. 6, 51 (1909).

[3] J. Biol. Chem. 12, 239, 245 (1912).

[4] Sherman, *loc.* cit., p. 57.

[5] "The Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino acids;" J. Biol. Chem., 10, 15 (1911). "The Quantitative Determination of Aliphatic Amino Groups," *Ibid.* 12, 275 (1912): "The Gasometric Determination of Aliphatic Amino Nitrogen in Minute Quantities," *Ibid.* 16, 121 (1913).

[6] "The Determination of the Composition of the Different Proteins of Ox and Horse Serum by the Method of Van Slyke." *Biochem* J., 8, 541 (1914).

" In carrying out the analyses of the different serum proteins the experimental conditions were kept as constant as possible. Two grams of material were boiled with 50 c.c. 20% hydrochloric acid and the rate of hydrolysis was followed by testing samples of the fluid at intervals in van Slyke's apparatus. The hydrolysis was continued for 16 to 18 hours.

" After hydrolysis of the protein the excess of hydrochloric acid was removed in vacuo and the hydrolyzed material washed into a 250 c.c. flask. Duplicate quantities of 25 c.c. of the solution were used for the determination of the total nitrogen, and 200 c.c. of the solution were used in all cases for analysis. The sum of the ammonia nitrogen, melanin nitrogen, total nitrogen of the filtrate, and the total nitrogen of the basis (the last named being determined in two parts) should be the same as the amount originally present, as determined separately in the 25 c.c. portions. * * *

" * * * In separating the diamino acids from the monoamino acids the same amount of phosphotungstic acid (15 grams) was used, and the precipitation was carried out at a volume of 200 c.c. in all cases. After the addition of the phosphotungstic acid the mixture was heated on the water bath until most of the precipitate had redissolved and after cooling to room temperature the Erlenmyer flask was allowed to stand for 48 hours in icebox. The precipitate of the bases was washed with an ice-cold solution of phosphotungstic acid in hydrochloric acid, 120 to 140 c.c. of the solution being used.

" One of the more difficult of the estimations in the process is the determination of the total nitrogen of the bases, which is carried out on the same solution used for the determination of the arginine. In these analyses the practice was adopted of continuing the digestion for some time after the solution becomes clear (not colored) gently moving the fluid round the sides of the flask from time to time in order to wash down into the acid any particles which may have crept on to the upper parts of the digestion flask. The value of the histidine nitrogen is not determined directly but is calculated from the results of three other determinations, viz., the arginine nitrogen, the amino nitrogen of the bases, and the total nitrogen of the bases. As van Slyke points out these three values can all be determined accurately, and the duplicate analyses for the histidine should not vary by more than one per cent. of the total nitrogen.

" The following estimations were carried out on the solution of hydrolysed protein:

"(1) Total nitrogen was estimated in 25 c.c. of the original solution.

"(2) Ammonia nitrogen and melanin nitrogen were estimated in 200 c.c. of the original solution.

"(3) After removal of the ammonia and melanin the separation with phosphotungstic acid was carried out.

"(4) The solution of the bases was made up to 50 c.c. and the following estimations made:
- "(a) Cystine nitrogen in 10 c.c.
- "(b) Amino nitrogen in 2 c.c.
- "(c) Arginine nitrogen and total nitrogen of the bases in the same sample of 25 c.c.

"(5) The filtrate from the phosphotungstic acid precipitate of the bases was made to 100 c.c. and the following estimations made:
- "(a) Total nitrogen of the filtrate in 25 c.c.
- "(b) Amino nitrogen of the filtrate in 2 c.c."

TABLE VI

DETAILS OF OBSERVATIONS—NITROGEN DISTRIBUTION ACCORDING TO THE METHOD OF VAN SLYKE

Material	Quantity Taken Grams	Total N		Ammonia		Melanin		Cystine		Arginine		Total N of Bases			Amino N of Bases		Amino N of Filtrate		Total N of Filtrate		
		cc. $\frac{N}{10}$ acid per 25 cc.	g. N. per 250 cc.	cc. $\frac{N}{10}$ acid per 250 cc.	g. N per 250 cc.	cc. $\frac{N}{10}$ acid per 250 cc.	g. N per 250 cc.	g. Ba SO_4 per 50 cc.	g. N per 250 cc.	cc. $\frac{N}{10}$ acid per 125 cc.	g. N per 250 cc.	cc. $\frac{N}{10}$ acid per 125 cc.	cc. $\frac{N}{10}$ acid per 125 cc. Arginine Titration	g. N per 250 cc.	cc. N per 10 cc.	g. N per 250 cc.	cc. N per 5 cc.	g. N per 250 cc.	cc. $\frac{N}{10}$ acid per 62.5 cc.	g. N per 250 cc.	Total N. Recovered per 250 cc.
Normal (D)	2	16.83	0.2357	10.09	0.0141	3.38	0.0047	0.0146	0.0044	3.96	0.0222	16.44	20.40	0.0572	2.580; 18.3°; 761 mm	0.0372	5.325; 20.5°; 754 mm.	0.1499	28.74	0.1619	0.2380
Tetanus globulin (B)....	2	17.67	0.2475	13.03	0.0183	4.35	0.0061	0.0120	0.0036	3.69	0.0207	15.19	18.88	0.0529	2.595; 24.0°; 759 mm	0.0362	5.956; 25.0°; 758 mm	0.1647	31.20	0.1748	0.2521
Diphtheria globulin (A)	2	18.01	0.2523	13.96	0.0196	5.01	0.0070	0.0137	0.0041	3.85	0.0216	15.16	19.01	0.0533	2.559; 24.0°; 763 mm.	0.0359	5.894; 21.5°; 762 mm.	0.1641	31.17	0.1747	0.2546

Table VI contains a record of the actual observations in the analyses, the volumes given in the headings referring in every case to the amount of the original solution corresponding to the volume actually employed as explained above. Three preparations were analyzed; normal serum (D), tetanus globulin (B), and the more active diphtheria globulin (A).

The results of the analyses given in Table VI are presented in Table VII as percentages of the nitrogen in the different forms in terms of the total nitrogen. The first row gives the resulst for normal serum, the second for the more active diphtheria globulin, and the third for the tetanus globulin. In the next three rows are quoted some results from Hartley's paper for the whole protein of horse serum, and then for the euglobulin obtained from this serum by two methods, the ammonium sulfate method and Panum's method. Where two series of results are given, their mean was taken.

No very marked differences in the compositions of the different preparations appear from this table. At the present time, the only points to which attention may be called are the higher values for cystine nitrogen in the tetanus and diphtheria globulins compared to the globulins given by Hartley, although the normal whole proteins show differences in the same direction; that is, a higher value was found for the cystine nitrogen in normal serum in this work than was found by Hartley in total protein. The histidine nitrogen was, however, distinctly lower for the tetanus and the diphtheria globulins than for any other of the preparations. Also the non-amino nitrogen was slightly higher for them than for the other normal substances.

TABLE VII

Results of Analyses Expressed in Percentages of Total Nitrogen

Material	Ammonia N	Melanin N	Cystine N	Arginine N	Histidine N	Lysine N	Amino N. of Filtrate	Non-Amino N. of Filtrate	Total N. Recovered
Normal Serum (D)	6.0	2.0	3.0	10.8	3.7	11.1	61.4	3.0	101.0
Tetanus-globulin (B)	7.4	2.5	2.5	9.7	2.3	11.0	64.4	2.1	101.9
Diphtheria-globulin (A)	7.7	2.8	3.3	9.8	2.0	9.1	64.2	2.1	101.0
Hartley's Whole Protein	7.3	1.6	2.1	9.5	5.6	12.8	60.0	2.0	100.9
Euglobulin (NH_4) SO_4 Method	7.9	2.3	1.7	8.3	5.5	10.0	62.2	2.2	100.1
Euglobulin Panum's Method	8.0	2.3	1.8	9.4	5.2	10.1	61.0	1.8	99.7

STUDIES IN PREPARATION OF DIPHTHERIA TOXIN MEDIA

(Analyses of Nutrient Materials)

Edwin J. Banzhaf and Louis J. Hirshleifer

About ten years ago, one of us studied the earlier published methods of media preparations, and evolved from them a medium which was adopted in most laboratories. The results obtained were fairly satisfactory in this and in other laboratories, producing a toxin with an M.L.D. ranging from 0.005 to 0.001 of a cubic centimeter. About a year ago, for some still unaccountable reason, the accumulation of toxin in the medium suddenly dropped to an M.L.D. ranging from 0.02 to 0.01 of a cubic centimeter. Other laboratories met with the same difficulties at about the same time. We were unable to increase the accumulation of toxin by any published methods of medium preparation or combines; neither could we by incorporating various commercial peptones singly or combined nor by using various ages of meats from " Bob " veal to beef, whether fresh or cold storage. The growth of the diphtheria bacilli on these media was much more luxuriant than the usual growth when the accumulation of toxin was satisfactory.

As a preliminary to an extensive study for obtaining a suitable and uniform medium for the accumulation of toxin, the following comparative analysis of commercial peptones was undertaken:

THE ANALYSIS OF PEPTONES

Numerous analyses of peptones and meat extracts have been published, but they have little value, and the results are useless for comparison, unless the exact methods of analysis are specified.

Some of the earlier methods may be mentioned:

J. Bruylant[1] described a method for analyzing meat extracts based on fractional precipitation by alcohol. Thus gelatin is thrown down by 40 per cent. alcohol, albumoses by 80 per cent. alcohol, peptones by 93 to 94 per cent. alcohol. The method is inaccurate because variations in the details of preparation of the meat extracts affect the results.

Rideal and Stewart[2] based their method on the fact that aqueous solutions of proteins are precipitated by chlorine. Allen[3] used bromine instead.

Stutzer[4] and also Mallet[5] separated the peptones from meat extracts, etc., by means of phosphotungstic acid. But neither the bromine nor the phosphototungstic acid method of separation of the peptones are sharp, and the results do not agree as much as could be desired.

Pick[6] separated Witte's peptone by fractional saturation with ammonium sulphate. The fractions were further divided according to their solubility in alcohol. Zunst[7] used zinc sulphate. This is preferred to ammonium sulphate, for the filtrates and precipitates can be directly applied to the estimation of nitrogen.

But, according to Schryver,[8] "The quantitative separation of a digest by means of salt precipitation must be regarded as an empirical process, and it is inadvisable to regard the various fractions to which the names of proto albumose, deutero albumose A, deutero albumose B, etc., have been assigned, as definite chemical products."

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[1] J. Pharm. Chim., 1897, p, p. 515.
[2] Analyst, 1897, 22, p. 228.
[3] Analyst, 1897, 22, p. 258.
[4] Z. anal. chem., 34, p. 568 (1895).
[5] U. S. Dept. of Agr. Bur. of Chem. Bul. 54.
[6] Zeitsch, Physiol. Chem., 1897, 24, p. 246; 1899, 28, p. 219. Beitr. Physiol. Path. Chem., 1902, 2, p. 481
[7] Zeitsch. Physiol. Chem., 1899, 28, p. 132. Beitr. Physiol. Path Chem., 1902, 2, p. 435.
[8] Allen, Comm. Org. Anal., 4th Ed, Vol VIII, p. 483.

Other reagents for the separation of the nitrogenous substances from meat extracts and allied products have been proposed and used at various times, such as formaldehyde, chromic acid, potassio-mercuric iodide, etc.

However, the methods described below for the analysis of the peptones, are at present considered as satisfactory. Again, inasmuch as the aim with the various peptones is to obtain comparative results, the methods of analysis need not be absolute in themselves, provided the same details in manipulation are followed throughout the series. Accordingly, proteoses are defined as products separated by complete saturation with zinc sulphate, and peptones as products which under certain conditions are precipitated together with the proteoses by Tannic Acid and Salt as developed by Bigelow & Cook.

Methods of Analysis

Total Nitrogen—This was determined by the usual Kjeldahl method on one-quarter gram sample. All nitrogen determinations were made according to this method.

Insoluble Nitrogen—2 grams sample dissolved in 10 c.c. water and boiled 5 minutes. Filtered and precipitate washed. Determined the nitrogen of the precipitate.

Proteose Nitrogen[9]—To aliquot parts of filtrate of insoluble nitrogen (equivalent to one-half gram sample) added 1 c.c. 50 per cent. sulphuric acid and saturated with solid zinc sulphate. Heated on water bath for a few minutes and allowed to stand over night. Filtered and washed precipitate with saturated solution of zinc sulphate (slightly acidified with sulphuric acid). Determined the nitrogen of precipitate.

Non-Protein Nitrogen[10]—The method is essentially as developed by Bigelow & Cook, 1 gram sample dissolved in 20 c.c. water in a 100 c.c. flask, mixed with 50 c.c. of 30 per cent. sodium chloride solution and allowed to stand in ice box 1 hour. Added 30 c.c. of 24 per cent. solution of Tannin (at ice-box temperature), mixed and diluted to mark. Allowed to stand in ice-box over night. Filtered and determined the nitrogen on 50 c.c. of the filtrate. Multiplied by 2 = non-protein nitrogen in 1 gram sample.

Peptone Nitrogen[10]—Total nitrogen — (insoluble nitrogen + proteose nitrogen + non-protein nitrogen) = peptone nitrogen.

Since peptone nitrogen is determined by difference, any errors in the other nitrogen determinations, accumulate on the peptone figure.

The attempt was made to determine peptone directly by treating the zinc sulphate filtrate after removing the proteoses with bromine.[11] The amounts of nitrogen obtained with some peptones were about half as much as by the Tannin-Salt method, whereas with other peptones more nitrogen was obtained.

It was also suggested to remove the zinc sulphate from the filtrate (free from proteoses) with hydrogen sulphide, and then precipitate out the peptone with strong alcohol. But this was found to be impracticable on account of the large amount of zinc sulphate in solution.

Amino Acids, Colorimetric Method[12]—This is based on the blue color obtained by the reaction of the alpha-amino acid nitrogen with "nin hydrin" in presence of pyridine, as developed by Harding & MacLean.

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[9] Allen, Comm. Org. Anal., 4th Ed., Vol. VIII, pp. 300, 483.

[10] Jo. Am. Chem. Soc., 1906, 28, p 1485.

[11] Sherman Methods of Org. Anal., 1912, p. 319.

[12] Jo. Bio. Chem. XX, 3, p. 217.

0.1 gram sample dissolved in 40 to 120 c.c. water, depending upon the probable amount of amino acids, and made neutral to phenolphthalein; 1 c.c. of the solution in a test tube was mixed with 1 c.c. of 10 per cent. pyridine and 1 c.c. of 2 per cent. solution of "nin hydrin" and heated in boiling water for 20 minutes. At the end of that time the test tube was removed, cooled, diluted to 100 c.c. and the color compared with that of the standard in a Duboscq Colorimeter.

The standard is prepared by dissolving 0.3178 gms. alanine in 1,000 c.c. 1 c.c. of solution was heated for 20 minutes in boiling water, with 0.5 c.c. of 10 per cent. solution of pyridine and 1 c.c. of 1 per cent solution of "nin hydrin." Cooled and diluted to 100 c.c. Equivalent to 0.05 mgm. N_2. The pyridine solution used was tested by titration by the method of K. E. Schulze.[13] The alanine was checked by the Van Slyke apparatus yielding 15.71 per cent. nitrogen (Theoretical per cent. N_2 for alanine=15.73 per cent).

Amino Acids, Van Slyke's Method[14]—For amino acids, the Micro Van Slyke Apparatus was used; 2 grams sample dissolved by heat, filtered and made up to 20 c.c. Used 2 c.c. of solution for each determination (equivalent to 0.2 grams sample).

Acidity—One gram sample dissolved in 75 c.c. water in a casserole, brought to a boil, added 1 c.c. phenolphthalein, and titrated with N-10 potassium hydroxide, till first appearance of pink.

Ash—Three grams sample ignited to ash in platinum dish.

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[13] Berichte, 20 (1887), p. 3391.

[14] Jo. Bio. Chem. IX, p. 185; XII, p. 275; XVI, p. 121.

Summary on Analysis of Peptones

	Total Nitrogen %	Ins. Nitrogen % of T. N.	Proteoses Nitrogen % of T. N.	Peptone Nitrogen % of T. N.	Non-protein Nitrogen % of T. N.	Amino $AcidN_2$ % of T. N. Colorimetric Meth.	Amino Acid Nitrogen % of T. N. Van Slyke Method	Ash %	Acidity c.c.
I	15.29	1.19	62.07	21.55	15.19	7.3	11.80	2.11	2.8
II	16.07	3.11	56.80	28.25	11.84	6.2	10.87	2.35	2.9
III	16.10	4.13	58.08	26.84	10.95	6.9	11.52	1.66	3.1
IV	16.24	4.18	57.06	27.56	11.20	6.7	11.14	1.80	2.9
V	15.23	2.75	59.19	23.72	14.34	7.2	12.81	2.25	2.4
VI	16.16	2.55	59.25	27.29	10.91	6.5	11.28	2.20	2.8
VII	13.16	1.27	5.74	46.62	46.37	16.4	23.94	12.73	9.9
VIII	15.68	1.02	43.21	40.60	15.17	5.0	9.17	3.45	1.9
IX	13.94	1.35	8.83	51.26	38.56	11.5	18.14	8.92	6.1
X	9.86	5.91	14.77	49.78	29.54	8.7	16.87	6.56	4.5

THE REDUCING ACTION OF DISTILLATES FROM CERTAIN CARBOHYDRATES ON DISTILLATION WITH STEAM AND FROM ALKALINE SOLUTION

James P. Atkinson

Several years ago a sample of water sent to this laboratory for analysis yielded extraordinarily high figures for free ammonia with the Nessler reagent. On further examination it was found to contain a reducing sugar.

Experiments were made to determine whether an alkaline solution containing glucose would yield under the conditions prevailing in water analysis a substance or substances that would react with the Nessler reagent similarly to ammonia.

Commercial glucose was dissolved in ammonia free water and the solution made alkaline with sodium carbonate. The distillate at first produced a greenish yellow color with Nessler's reagent the following tubes increasing in depth until precipitation of the reagent occurred.

This interesting result led us to try the effect of distillates of solutions of other carbohydrates including filter paper after distillation in both ammonia free distilled water, and in water made slightly alkaline with sodium carbonate.

The preliminary experiments were carried out on laboratory samples of unknown purity excepting the filter paper which in all experiments was S. & S. No. 589.

The final results given in the table were made on as pure substances as could be purchased. They correspond, however, to the first results obtained on the laboratory samples.

The procedure was exactly as it would be carried out in an examination of water for free ammonia, except in those distillations where sodium carbonate was omitted. Results were not affected whether block tin condensers or apparatus made entirely of glass were used.

Beside the reaction with Nessler's reagent the effect of the distillates on Fehling's solution and ammoniacal silver was tested.

The color produced by the addition of Nessler reagent to the distillates from the sugars alone is a greenish yellow and cannot be readily confused with the true ammonia reaction. When ammonia compounds are mixed with the sugars the greenish tinge is masked.

*V. Lippmann in Chemie der Zuckerarten, III Auflage, gives references to the reactions of various carbohydrates on treatment with alkalies. Certain reducing compounds are formed which might account for these reactions notably formic acid, aldehydes and ketones.

Laevulose, Maltose, Mannose and Dextrin in 1 gram quantities gave distillates when distilled without sodium carbonate yielding a yellow color with Nessler reagent.

When alkaline potassium permanganate was substituted for sodium carbonate the reducing reactions were more marked.

Since some of these substances shown in the table, notably cellulose, starch and reducing sugars from vegetable matter occur in surface water such as rivers, brooks, lakes and reservoirs, it is conceivable that they may have an influence on the depth of color in the ammonia readings and it is the purpose of this contribution to call attention to this possibility.

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* See pages 1212 and 1237 for cane sugar, 1475 for maltose, 1546 for lactose, 328 for glucose.

*REACTIONS OF DISTILLATES OF CERTAIN CARBO-HYDRATES

Carbohydrates	Amount	Fehling Sol.	Amount	Silver Nitrate	Amount	Nessler Reaction	Odor
Saccharose, Kahlbaum.........	5 grams	Faint reduction	5 grams	Faint reduction	1 gram	No reaction with 1st distillates, later distillates cloud reagent and give yellowish color.	
					5 grams	Similar reactions.	
Lactose E. & A. Highest purity.	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	1st distillates gives strong yellow color and later ones reduce the reagent to metallic mercury.	Caramel
Traubenzucker, Kahlbaum......	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	Caramel
Laevulose crystals, Kahlbaum..	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	Caramel
Maltose, Kahlbaum............	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	Caramel
Mannose, Kahlbaum...........	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	......
Dextrin, C.P. by alcohol, E. & A.	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	Caramel
Soluble starch, E & A..	5 grams	Readily reduces	5 grams	Readily reduces	1 gram	Reactions similar to lactose.	Caramel
Filter paper (cellulose) S. & S, No. 589. Freshly washed with hydrochloric acid and ammonia free water..................	....	Reduces	....	Reduces	....	Reactions similar to lactose.	Caramel

*Solution made alkaline with sodium carbonate.

When Nessler reagent is used for measuring small quantities of ammonia in determinations other than in water analysis, where there is a chance of carbohydrates being present as in urine and blood these reactions should be considered.

The product of decomposition causing these reactions has not been identified, though some work has been done upon it.

The analytical work, the final results of which only have been given, was carried out during several years by Messrs. Miller and Rosencrans and Dr. C. H. Jouet of the laboratory.

THE RAPID DETERMINATION OF SMALL QUANTITIES OF HEROIN

REGINALD MILLER

(*Reprinted from Am. Jour. Pharmacy, June,* 1915. *Vol.* 87, *No.* 6, *p.* 248)

This laboratory frequently receives samples of drugs for analysis among which are heroin and cocaine.

In many cases the total quantity of heroin does not exceed ¼ grain mixed with varying amounts of milk sugar and sometimes cocaine. To determine this quantity of heroin by the extraction method is extremely difficult, particularly if cocaine is present as well as heroin. A rapid method by which small quantities of heroin could be determined with reasonable accuracy was sought to facilitate these examinations.

The following method was used and found, in the absence of morphine or any other interfering substance, to be sufficiently accurate and to possess the additional advantage of being rapid.

After the presence of heroin and absence of morphine or other interfering substances are ascertained, a weighed amount of the powder is taken sufficient to contain from 1/50 to 1/20 grain of heroin. This can be roughly determined by the qualitative reactions. It is placed in a Nessler tube, and 1 Cc. of a one per cent. solution of sulphuric acid is added, and then 3 Cc. of a solution consisting of 600 Cc. of commercial sulphuric acid, 300 Cc. of water, and 25 Cc. of a 40 per cent. formaldehyde solution. This reagent will produce a coloration, varying from a yellowish straw for 1/150 grain to a deep cherry red for 1/5 grain of heroin, depending upon the length of time the reaction is allowed to proceed and the amount of heroin present.

A series of standard tubes are prepared, containing 1/50, 1/40, 1/30, 1/20, and 1/15 grain of heroin respectively (or any other suitable quantity from 1/150 grain to 1/5 grain), each of which is treated with the reagent in the same manner and at the same time as the sample. The reaction is allowed to proceed for 10 or 15 minutes in all the tubes, when the coloration in the tube containing the sample is compared with the colorations in the standard tubes. The standard tube which has the same intensity of color as the sample contains the same amount of heroin, and from this figure we compute the total amount of heroin in the powder under examination.

If, however a mixture of cocaine and heroin is submitted, we determine the heroin and cocaine as follows:

The substance is extracted in the regular manner by the immiscible solvents, and the residue of heroin and cocaine is weighed, and dissolved in a *known* amount of 1 per cent. sulphuric acid, so that 1 Cc. of the solution will contain between 1/100 and 1/20 of a grain of heroin. One Cc. of this solution is put into a Nessler tube and treated with 3 Cc. of formaldehyde sulphuric acid solution. The color produced is compared and measured as described. The standard tube containing the same intensity of color is used as the basis for computing the amount of heroin in the weighed residue, and the difference between the heroin and weight of residue obtained by the immiscible solvent represents the cocaine.

The following table illustrates the sensitiveness and the limitation of the method. A period of 15 minutes after the addition of the reagent (formaldehyde sulphuric acid) was allowed in each of the following cases before a note of color produced was made:

1/200 grain of heroin: slight coloration produced.
1/100 grain of heroin: light reddish-yellow color produced.
1/50 grain of heroin: red-yellow color produced.
1/45 grain of heroin: deeper than 1/50 of a grain.
1/40 grain of heroin: deeper than 1/45 of a grain.
1/30 grain of heroin: much deeper than 1/40 of a grain.
1/20 grain of heroin: very much deeper than 1/30 of a grain.
1/10 grain of heroin: cherry-red color produced.
1/5 grain of heroin: deep cherry-red color produced.

I have found that a difference of 1/450 of a grain of heroin will produce an appreciable change in color when 1/50 of a grain is used, and that a difference of 1/100 of a grain of heroin will produce a very perceptible change in color when 1/20 of a grain of heroin is used.

I prepare these standards by making up several solutions of heroin in one per cent. sulphuric acid solution, and then pipetting by means of a pipette graduated in 1/100 Cc.

One solution is prepared containing 1/5 grain of heroin per Cc. Another solution is prepared containing 1/10 grain of heroin per Cc. A third is prepared containing 1/20 grain of heroin per Cc. A fourth solution is prepared containing 1/40 grain of heroin per Cc.

THE TREATMENT OF HUMAN RABIES WITH QUININ AND WITH CARBOLIC ACID

Frank S. Fielder

Since the preventive treatment of persons bitten by rabid animals has come to be widely practised, rabies in the human being is a rare disease, yet in a large city like New York a few cases occur every year (in 1911 eleven cases; in 1912 six cases; 1913 and 1914, eight cases each; 1915, one case).

Most of the persons who develop rabies have received no preventive inoculations,[3] yet occasionally the disease occurs in spite of such treatment, the gross incidence among many thousands of persons treated by different antirabic institutes all over the world averaging about one-half of one per cent.

So far as we positively know, the disease, once developed, is always fatal. In the few cases of recovery that have been reported, the diagnosis has been open to serious doubt. Attention has been centered upon the prevention of the disease by one or other of the accepted methods (Pasteur, Hogyes, Harris or Cumming), and very little effort has been made to find a specific treatment of developed rabies. Yet in view of the facts that the balance of evidence leans toward the protozoan nature of rabies, and that certain protozoan infections are amenable to cure by specific drugs, it would seem worth while not to abandon hope of finding some drug that would kill the parasite in vivo without harm to the patient.

In 1913 Moon[1] reported the recovery of three dogs treated with massive doses of quinin after the development of symptoms of rabies. Cumming[2], and Frothingham and Halliday[3], using rabbits, failed to confirm Moon's results. Krumweide and Mann[4] (see also page 56 of this volume) found that quinin had no effect in shortening the incubation of rabies in rabbits. Of four dogs inoculated intracerebrally with rabies virus and subsequently treated with quinin per os and subcutaneously, one showed a prolonged period of incubation and one a remission in the symptoms, in each case however not beyond the limits of variations known to occur in the natural course of the disease. The dogs all died clinically rabid, their brains showed Negri bodies, and guinea pigs inoculated subdurally from them all died of rabies and also showed Negri bodies.

Although these experiments failed to confirm the results reported by Moon, the writer decided to try the quinin treatment upon a human case at the first opportunity.

Treatment of Human Rabies with Quinin

In October, 1913, D. L. Harris[5] reported the case of a man who showed symptoms strongly resembling rabies, and who was treated with quinin and urea hydrochlorid, receiving 90 grains intravenously, with prompt recovery. A careful reading of this report leads to the suspicion that this man did not have rabies, for the following reasons among others: There was no evidence that the dog that bit him was rabid; the man managed to drink enough whiskey to get intoxicated before he entered the hospital for treatment, and his symptoms improved too rapidly. Two and one-half hours after treatment was begun, when he had had but two intravenous injections of 15 grains each, he drank six ounces of milk without difficulty. Later that night and on the following day he could eat and drink, and his recovery was complete within a day or two. On the other side of the argument, it must be admitted that the patient's symptoms as described were so characteristic that, had he died, one would have felt certain he died of rabies, even in the absence of post-mortem confirmation of the clinical diagnosis.

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[1] Jour. Infect. Dis., 1913, 13, p. 165.
[2] Jour. Infect. Dis., 1914, 15, p. 205.
[3] Jour. Med. Research, 1914, 30, p. 275.
[4] Jour. Infect. Dis., Vol. 16, No. 1, Jan., 1915.
[5] Jour. A. M. A., Vol. 61, No. 17, Oct 25, 1913, p 1511.

On October 26, 1913, two days following the publication of Harris's report, an opportunity of trying the quinin treatment upon an undoubted case of human rabies presented itself.

Case 1. The patient, A. A., age seven years, was bitten rather deeply on the index finger on October 4, 1913. The wound was improperly cauterized at a dispensary with nitrate of silver. The dog was killed and examination of the brain at the Research Laboratory showed Negri bodies present. Three days later the boy appeared at the antirabic clinic of the Department of Health and the Pasteur treatment was begun under the direction of the writer. The child was irregular in attendance and received only fourteen treatments in eighteen days, the last one on October 24th.

Symptoms of rabies began on October 25th, and he remained at home. No report of his illness was made to the clinic. On the following afternoon he was taken to Bellevue Hospital, where the diagnosis of rabies was made. On the morning of October 27th the writer was notified by the House Physician, who had just read Harris's article. Although the boy had then been ill for forty-eight hours, it was determined to try the quinin treatment. At this time all the classical symptoms of the disease were present: extreme nervousness, complete inability to swallow fluids, and great fear of attempting to do so, clonic spasms of the muscles of the throat and face and occasional general convulsions.

At 11 A. M. ten grains of quinin and urea hydrochlorid were given intravenously in 10 c.c. of sterile water. Three hours later lumbar puncture was performed, clear fluid withdrawn, and seven grains of quinin were given intraspinally. This was followed by an alarming collapse, which lasted about two hours. Later in the afternoon another dose of ten grains was given intravenously. The drug had no apparent effect upon the symptoms of the disease, which proceeded as usual through convulsions and paralysis to death from exhaustion on the morning of October 28, three days after the onset of the symptoms and less than twenty-four hours after the quinin treatment was begun. Necropsy showed Negri bodies in the brain; animals inoculated with the brain material developed rabies, and Negri bodies were found in their brains.

It was felt that this case was not a fair test of the value of quinin in rabies, because the treatment was begun so late in the disease.

Case 2. During the Spring of 1914 the writer was called to see a case in the Polyclinic Hospital, which presented symptoms very suggestive of rabies. The patient, a young cabaret singer, said she had been bitten by her own dog five weeks before at a time when the animal was sick. She had given the dog away immediately after the bite, and did not know its subsequent history. She appeared at the throat clinic of the hospital complaining of spasms of the throat and inability to swallow. Upon examination several hours later, she presented a very fair clinical picture of rabies: abundant ropy saliva, convulsive movements of the throat and head, marked hydrophobia and total inability to swallow anything. Examination of the throat, made with difficulty, disclosed only congestion of the fauces, pharynx and larynx. She was extremely nervous and excited.

Although the writer suspected hysteria, the symptoms were so nearly typical of rabies that it was decided to administer quinin. Ten grains of quinin and urea hydrochlorid were given intravenously at 5 P. M. When seen three hours later she was so much better that no more quinin was given and a positive diagnosis of hysteria was made. One of the ambulance surgeons thought he recognized the woman, and a search of the ambulance records disclosed the fact that she had been brought into the hospital three times before with different manifestations of hysteria. She was discharged the next day, cured.

This case appears to be somewhat similar to the one reported by Harris, and is cited to show how closely hysteria or some other psychosis may simulate true rabies.

Case 3. In the following case the quinin treatment was fully tried out, but failed to save the patient's life. Grace P., age 13 years, was bitten upon the right wrist by a cat October 19, 1913. The wound, which was a slight one, was treated at once with tinct. iodin by the family physician, Dr. Theo. L. Vosseler. The cat was killed and its brain examined at the Research Laboratory, Department of Health. Negri bodies were found. On October 20th the wound was recauterized at the laboratory with pure nitric acid, and Pasteur treatment, supplied by the Department of Health, was given daily by Dr. Vosseler for twenty-one days. The patient and her family denied any history of bites from animals or any other opportunity for rabic infection between this time and the date of the development of rabies, which occurred one year after the completion of the Pasteur treatment.

On November 1, 1914, the child complained of pain in the right wrist. This lasted four days, extending from fingers to shoulder, and then subsided.

During the night of November 7, 1914, following an afternoon of very active play, she began to have pharyngeal and laryngeal spasms, with some difficulty in swallowing. The next noon Dr. Vosseler was called, and finding the above symptoms more pronounced, made a diagnosis of rabies. Dr. Frederick Tilney was called in consultation at 8 p. m. and confirmed the diagnosis. Late that evening the writer was summoned and saw the patient with Drs. Vosseler, Tilney and Thunig. The child then complained chiefly of difficulty in breathing. She was unable to swallow water, and spasms of the throat and face muscles were easily elicited by blowing a puff of cool air upon the face.

It was decided to administer quinin in massive doses, beginning with an intraspinal injection. The risk of this method was fully explained to the parents and accepted by them. At one a. m., November 9th, 20 grains of quinin and urea hydrochlorid in 10 c. c. boiled water, were given intraspinally by Dr. Tilney, after removing 18 c. c. of clear cerebro-spinal fluid which was saved for laboratory tests.

Within ten minutes the child complained that she could not move her lower limbs. She soon stopped breathing and paralysis of all the muscles of the body, except those supplied by the cranial nerves, occurred. For a few minutes the heart action was weak, and the child appeared about to die. The heart was stimulated and the head of the bed raised to keep the quinin, so far as possible, in the lower part of the canal. Artificial respiration was performed for about an hour until a pulmotor could be procured, which soon (in nine minutes) restored voluntary breathing. Consciousness was retained throughout, but the child could not speak. By 3 A. M. ability to speak and to move the muscles of the arm and trunk had returned. Complete motor and nearly complete sensory paralysis of the lower extremities persisted until death.

It was evidently too dangerous to give further intraspinal injections, and the remaining doses of quinin were given intravenously as follows:

November 9, 5 a. m., 20 grs.; 10 a. m., 20 grs.; 2 p. m., 20 grs.
November 10, 1 a. m., 20 grs.; 12 m., 15 grs.; 5 p. m., 20 grs.
November 11th, 11 a. m., 20 grs.; 5 p. m., 20 grs.
November 12th, 1 a. m., 20 grs.

Thus, a total quantity of 195 grains was given in seventy-two hours, in ten doses. The intraspinal dose was dissolved in 10 c. c. of boiled water, the first five intravenous doses in 6 to 9 c. c., and the last four in 12 c. c. of normal salt solution. Owing to the local irritation caused by the quinin, the injection had to be given in a new place each time, and all available veins of the upper and lower limbs were used.

Without describing the symptoms in too great detail, it may be stated that the only beneficial effects of the quinin seemed to be a reduction in the amount of conscious suffering, and probably some prolongation of the course of the disease, since the child lived six days. On November 9th, there were convulsions of the face, head and upper extremities, and later in the day a few general convulsions, but these were not as severe as are usually seen in rabies. Owing to the paralysis of the lower extremities caused by the intraspinal dose of quinin, there were no convulsions below the waist-line at any time. On the 10th, from 2 a. m. until noon, there were severe throat spasms, general convulsions and active delirium at times. The child could not swallow either liquids or solids, except on the 12th when she took six ounces of water with difficulty. From November 11th on, she could not see, and on the 12th she became comatose, after which the spasms of throat, face and arms became less frequent and less severe. Some of the injections of quinin were followed by a marked reduction in the frequency and force of the heart action. On November 11th, the leucocytes were 35,000 with 86 per cent. polynuclears; on the 13th, 13,500. The urine was drawn by catheter, and on the day of death it contained 25% albumen by volume and gave a prompt Fehling reaction for sugar.

Aside from the injections of quinin, the treatment consisted in sedatives, enteroclyses, hypodermoclyses, rectal feeding and cardiac stimulation by adrenalin hypodermatically.

Gradually the patient's strength became exhausted and finally death occurred suddenly from respiratory paralysis on the evening of November 13th, six days after the onset of the symptoms. Necropsy, November 14th. Negri bodies were found in the brain, and guinea pigs inoculated subdurally from this developed rabies in two weeks and Negri bodies were found in their brains. Guinea pigs inoculated subdurally with the cerebrospinal fluid did not develop rabies, thus confirming the customary experience that the spinal fluid of persons suffering from rabies is usually not infective.

This case seems to show that the administration of quinin intraspinally is extremely dangerous, that it is fairly well borne intravenously, but that not much can be expected of it in rabies, by either of these methods of administration.

TREATMENT OF RABIES BY SUBCUTANEOUS INJECTIONS OF CARBOLIC ACID

In the autumn of 1913, Haberlin[6] published the details of a case clinically resembling rabies, treated successfully by subcutaneous injections of dilute solutions of carbolic acid. His patient, a dog-catcher, of alcoholic habit, was bitten on the calf of the leg by a dog that was suspected of being rabid. This dog bit another man, who developed rabies five weeks later and died. There was no report of post-mortem examination of either dog or man.

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[6] N. Y. State Jour. Med., Sept., 1913.

Two days after the man's death, Haberlin's patient, who had been taciturn, morose and depressed for three or four days, began suddenly in the early evening to have spasms of the pharynx and larynx and complete inability to swallow, accompanied by extreme nervous agitation and followed by violent general convulsive seizures, alternating with periods of comparative calm. He drooled and spat saliva continually. The laryngeal muscles became tetanic to a degree that seemed to threaten suffocation, and the labored attempts at expiration produced a harsh, brassy cough that somewhat resembled the so-called rabid "bark."

A diagnosis of rabies was made, and it was decided to administer carbolic acid according to the technic of Bacelli in cases of tetanus. At 8.30 P. M. 10 c. c. of 1 per cent. aqueous solution of phenol were injected subcutaneously. One-half hour later 10 c. c. of a 2 per cent. solution were injected, and repeated in an hour. One hour after that there was a very perceptible improvement in the patient's condition. Hourly doses of the 1 per cent. solution were now resumed and continued for ten hours, a total of eleven doses of 1 per cent. and two doses of 2 per cent. solution, equivalent to one and one-half grams of pure phenol.

At 2 A. M., six hours after beginning treatment, the patient became somnolent, and when roused could swallow readily. At this time he stated that he was now conscious of his surroundings for the first time since the early evening, and when reminded of his previous actions and statements he denied any recollection of them. His subsequent history was uneventful. On the fourth day the patient was out of doors, and on the sixth day he resumed work.

As in Harris' case, this man's recovery was so prompt that it seems hard to believe that he really had rabies, yet the clinical symptoms were characteristic and closely resembled those of the other patient who was bitten by the same dog, and who died of the disease.

It was determined to treat our next case of rabies by the above described method.

Case 4. Nathan J., aged two and one-half years, bitten November 10, 1914, by a stray dog, which was not captured. Twelve extensive but superficial wounds upon the face. No cauterization by the ambulance surgeon who first saw the case. Three days later he was brought to the Bronx Antirabic Clinic of the Department of Health. There the wounds were not cauterized, since three days had already gone by, and the scarring that would have followed the application of nitric acid to such extensive face wounds did not seem justifiable in the absence of proof that the dog was rabid. Two other children were also bitten on the face by the same dog. All three were given a full course of Pasteur antirabic treatment, the adult dose being used.

On December 9, 1914, six days after the completion of his treatment, Nathan J. became feverish and restless, and was seen by the family physician, Dr. J. J. Gurtov, who made a diagnosis of influenza. On December 11th the mother noticed that the child had difficulty in swallowing. She called the doctor again, and he, suspecting rabies, notified the writer who saw the patient in the afternoon of the same day. At that time the clinical picture was confusing. The child had a temperature of 104 deg. F., and was restless and easily frightened. He drank water without much difficulty.

There were no pharyngeal spasms. Blowing cold air upon his face caused fright, but no spasmodic twitchings.

The carbolic treatment was explained to the doctor and he was advised to begin it later in the evening if difficulty in swallowing increased, giving two doses of 2 c. c. of a 2 per cent. aqueous solution subcutaneously an hour apart, followed by hourly doses of 5 c. c. of 1 per cent. solution. At 11.30 P. M., the symptoms having increased, the treatment was begun, and continued at hourly intervals until 7.30 A. M. During this period the child was very wild at times, and would stagger and fall on attempting to walk. He had a few pharyngeal spasms, but could swallow warm fluids. The parents thought he could not see.

On December 12th the patient was seen by the writer at 9 A. M. As there was some rigidity of the neck, a lumbar puncture was performed and turbid fluid withdrawn (an unusual occurrence in rabies). Two more doses of 1 per cent. carbolic were given, and at 11 A. M. the child was removed to the Reception Hospital of the Department of Health. There, at 1 P. M., lumbar puncture was again performed. The spinal fluid was turbid and under pressure; 20 c. c. were withdrawn, and anti-meningococcic serum introduced. The fluid on examination showed abundant leucocytes but no organisms. Blood examination showed leucocytes 24,800, polynuclears 84 per cent., lymphocytes 16 per cent.

The carbolic injections were resumed and continued hourly. In all, the child received two injections of 2 c. c. each of 2 per cent. and fifteen of 1 per cent. solution, equivalent to about 11 grains of pure phenol.

Upon admission to the hospital the child was extremely nervous, barely closed his eyes, started at the least sound and had an anxious expression. There were intermittent twitchings of the muscles, and at times the whole body was involved. The right leg and arm were moved from place to place, but it was noticeable that the left leg and arm moved hardly at all. The reflexes were a little exaggerated. There was internal strabismus of the left eye and at times a convergent strabismus. The pupils were equal and reacted to light. Blowing cool air upon the face caused marked disturbance, either pharyngeal spasm or a movement of the arms as if to repel the disturber. Attempts to drink caused evident discomfort and slight spasm of the throat; later he was unable to swallow any fluids. He was conscious and would open his mouth when asked; he answered questions.

At 6.30 P. M. the patient had slight convulsions when the bed clothes were lifted or when a draught reached the face or body. The twitchings were intermittent until 7.30 P. M., when he passed into coma. Respirations became very slow, at one period as low as eight per minute. There were no more convulsions, and the child died at 8 P. M., December 12th, three days after the onset of his illness, and about thirty hours after the appearance of the first suspicious symptoms.

Necropsy was performed on the following day. Only the brain was examined. The pia was congested and cloudy; no evidence of meningitis Miscroscopic examination of the brain showed no definite Negri bodies on careful search, and the diagnosis seemed in doubt, especially in view of the finding of turbid spinal fluid, which, in rabies, is almost always clear. Two guinea pigs were inoculated subdurally with material from the child's brain. One of these died in twelve days and one in fourteen days, both having shown clinical evidences of rabies. Negri bodies in abundance were found in the brains of both animals.

In this case the carbolic acid injections evidently had no effect on the progress of the disease.

The other two children bitten by the same dog were given a second full course of antirabic treatment, beginning a month after the completion of the first course. Neither one developed rabies.

Conclusions—The cases cited, so far as can be judged from such a small number, show no evidence that either quinin or carbolic acid has any specific effect upon rabies.

The thanks of the writer are due to Drs. Vosseler and Gurtov, for permission to report their cases, and to Dr. Anna W. Williams and Miss Alice G. Mann, who made the laboratory studies in cases 1, 3 and 4.

PARALYSIS COMPLICATING PASTEUR ANTIRABIC TREATMENT

(With reports of seven personal cases, one terminating fatally, and six other previously unreported cases.)

Frank S. Fielder

In the vast majority of instances, the Pasteur antirabic treatment is neither accompanied nor followed by any complications of importance. The slight pain of the injections, some degree of local or constitutional reaction, occasionally a fairly marked urticaria, possibly a localized abscess if the physician be careless in the care of his syringe—these are all the discomforts that are likely to be met with.

There is one complication, however, the so-called " treatment paralysis," which has long engaged the attention of those in charge of antirabic institutes, and the true nature and cause of which are not fully understood.

This complication occurs with great rarity and usually terminates in recovery, so that it should be considered no bar to antirabic treatment when this is evidently needed, but the possibility of paralysis should always be explained to those in whom infection is doubtful, or who are inclined to insist upon being treated merely as a sentimental matter of precaution.

The symptoms vary from a slight degree of neuritis, with little or no motor involvement, to an acute ascending paralysis which may be fatal.

An analysis of the parts of the body involved in 37 of 40 cases reported by Remlinger[7] in 1905 is as follows:

Legs and sphincters	17 cases
Legs alone	6 cases
Legs, sphincter, arms, face	4 cases
Legs, sphincters, face	4 cases
Arms and face	1 case
Arms alone	1 case
Face alone	1 case
Legs, arms, sphincters and bulbar symptoms	1 case
Legs and face	1 case
Legs, sphincters and bulbar symptoms	1 case

Thus, in 37 cases, the legs were involved in 34, sphincters in 27, face in 10 and arms in 7 of the cases, while bulbar symptoms were present in only two.

The frequency of paralysis as a complication of antirabic treatment is variously estimated by different writers, but its real frequency is unknown, since many cases, especially the mild ones, are doubtless not reported.

Remlinger,[7] in 1905, collected 40 cases of paralysis out of 107,712 patients treated, two of these being fatal. Stimson,[30] in 1910, stated that later reports had brought the total up to 76 cases, with 4 deaths. Simon,[37] in 1913, collected data up to and including 1911, showing the occurrence of 100 reported cases among 217,774 persons treated, and mentioned 3 other cases, without indicating the number treated. In this series there were 19 deaths.

The incidence of paralysis, however, is evidently different under different methods of antirabic treatment. Simon's[37] figures showed 6 cases among 32,676 patients treated by the classical Pasteur method, 16 cases among 8,659 patients treated by the modified (more intensive) Pasteur method, and only 3 cases among 51,417 patients treated by the Högyes method, with dilutions of fresh fixed virus.

Hasseltine,[43] in a brief review of the literature since Simon's report, mentions cases reported by Higier,[34] Hudovernig,[35] Sterling,[36] Kozewalow,[41] Mucharinski, Abramov, Jochmann,[38] Goldberg and Oszesalski,[39] and Fabricius.[22] He (Hasseltine) adds four cases of his own, one of which was fatal, and states that he has knowledge of two other cases as yet unpublished. He quotes Park and Williams,[42] who state in their book on Pathogenic Microorganisms, edition of 1914, p. 585, that there have been seven cases of paralysis with 2 deaths among 6,850 cases treated by the Pasteur clinic of the New York City Health Department including the year 1913. Hasseltine thus brings the total up to 133, up to and including 1913, with 25 deaths.

Poor,[29] in 1909, reported 8 cases of neuritis without paralysis, and 4 of paralysis, one of which was fatal, among 2,300 patients treated by him at the New York City Health Department. The figures of Park and Williams, quoted above, include Poor's four cases of paralysis, and three of the cases, including the fatal one, which are reported in the present paper.

The writer's seven personal cases, together with the 6 other cases which are here first reported thus bring the total up to 143 cases with 25 deaths. This should be corrected to 142 cases with 24 deaths, since Poor's fatal case and that of Fabricius were the same.

THE MORTALITY among these published cases, 16.2 per cent., must not be taken as representing the true mortality incident to this complication of antirabic treatment, since it is certain that many mild cases are not reported, while it is likely that most of the severe and fatal ones have been published. Although the average mortality among published cases of paralysis is 15 to 20 per cent., this is reduced to a negligible figure, when compared with the total number of cases treated. Simon's[37] figures, 100 reported cases with 19 deaths among 217,774 patients treated at various institutes, shows a mortality of 19 per cent. among the cases developing paralysis, but so rarely does a case occur that the incidence of the disease in his series was only 0.048 per cent. and the mortality but 0.00812 per cent. of the total number of cases treated.

AS REGARDS PROGNOSIS, the cases may be roughly divided into three classes. The mild ones with transitory paralysis of face or limbs all recover. The acute paraplegias of the lower extremities with or without the involvement of the sphincters usually recover. Death, if it occurs, is likely to be due to cystitis or bed-sores after a prolonged illness. Remlinger[7] reported 2 deaths in 40 cases and Simon[37] 3 deaths in 24 cases of this type. The severest cases, presenting the clinical picture of an acute ascending paralysis of the Landry type, have a bad prognosis. Simon[37] reported 15 deaths among 36 such cases, a mortality of 41.6 per cent. The deaths in this last group occur within a few days from respiratory or cardiac paralysis or later, from inhalation pneumonia, cystitis or bed-sores.

THE TIME OF DEVELOPMENT OF SYMPTOMS is fairly characteristic. According to Simon[37] they usually make their appearance within 11 to 30 days after the bite, and between 10 and 20 days after the beginning of the injections. Only one-quarter of the cases develop after the completion of a full course of treatment, and most of these within seven days thereafter.

In the 13 cases reported in the present paper, the development of first symptoms of paralysis in relation to the date of the bite, and to the beginning of treatment is as follows: Writer's 7 personal cases: symptoms first appeared during treatment in 6 cases, and three days after its completion in 1 case. Average, 23 days after the bite and 17 days from beginning of treatment. Among the 6 other cases, symptoms first appeared during treatment in 4 cases. In 2 cases they appeared in 2 and 10 days respectively after treatment. Average, 26 days after the bite and 17 days from the beginning of treatment.

These figures tend to bear out Simon's statement that, whether counting from the date of the bite or from the beginning of treatment, the incubation is shorter than in most cases of rabies. This fact is important in view of the contention of some writers that the condition is one of true rabies caused by the bite, but rendered mild by the antirabic treatment.

The onset of the symptoms is usually acute, with pain and numbness of the affected parts, followed by muscular weakness. Prodromal symptoms if present are mild and consist only of malaise and nervous depression, with perhaps some restlessness, insomnia and anorexia.

Sex.—Males seem to be affected much more frequently than females. In Simon's[37] publication, among 84 cases there were 60 males and 10 females; sex of the remaining 14 unknown. Hasseltine's[40-43] 4 cases were all males. Of the writer's 7 personal cases 5 were males, and of the 6 additional cases reported, all were males. This preponderance of males is probably partly due to the fact that undoubtedly more males are bitten and thus more are treated. Unfortunately, available statistics do not state the relative proportion of males and females in the total number of cases treated.

Age.—Young children are rarely affected. Most of the cases occur in adults of varying ages. All of the 13 cases reported in this paper were adults between 23 and 62 years of age, the average being 41 years. Seven of the cases were in the thirties.

Predisposing Conditions.—It is stated that persons who are alcoholic, syphilitic or neurotic are more likely to be affected than those who have no such taint, but exceptions to this rule are seen fairly often. Paralysis occurs so rarely in proportion to the total number of cases treated that a special idiosyncrasy on the part of the patient must be assumed.

Pathology.—Jochmann,[38] in a careful study of a fatal case, found the lesions disseminated, but especially localized in the lumbar and cervical cord, and associated with a perivascular serous transudation and with marked oedema of the meninges of the dorsal and lumbar cord. Small foci of softening were present in the lumbar cord. The large ganglion cells of the gray matter had partly disappeared; some were atrophic and their chromatin had gone into solution. A mild round cell infiltration was noticeable throughout the whole cross-section of the gray matter. The same changes were found in the cervical cord, but to a much less degree. The brain findings were not described except that Negri bodies were not demonstrated.

Rabbits, rats and dogs were inoculated by Jos. Koch from the lumbar cord of this case. Some of the animals, after a long incubation, died of rabies. In two of the rabbits, Negri bodies were found in size and shape as they occur in inoculation rabies.

The serous exudation and meningeal œdema which occurred in the lumbar cord in this case would explain the otherwise puzzling clinical fact that, in spite of a most severe symptom-complex, such as complete flaccid paralysis of the extremities, colon, bladder, etc., the symptoms may begin to abate within a few days, and a rapid cure result.

Etiology.—This must still be considered as not definitely settled. The principal explanations advanced for the phenomena observed are:

(1). *Anaphylaxis,* due to repeated injections of foreign proteid contained in the rabbit cord employed.—This explanation was advanced by Harvey and McKendrick, and entertained by Müller. It is conceded that the Högyes method of treatment with diluted fresh fixed virus, in which the proportion of nerve substance is at a minimum, is far less often complicated with paralysis than is the Pasteur method, in which the amount of nerve tissue is much larger. The following summary from Simon[37] shows this:

TABLE I

Incidence of Paralysis Under Different Methods of Antirabic Treatment

	Number of Cases Treated	Cases of Paralysis	Proportion
Classical Pasteur Method	32,676	6	1:5446
Modified Pasteur Method	8,657	16	1:541
Högyes Method	51,417	3	1:17139

If the foreign proteid material in itself were the cause of "treatment paralysis," the percentage of cases occurring under the classical and modified Pasteur methods should be approximately the same, since they contain about the same amount of nerve substance, differing only in the strength of material used. Yet, as shown by the above table, paralysis occurs ten times as often under the modified (intensive) as under the classical Pasteur method.

Another point against the anaphylaxis theory is the fact that paralysis has not been produced in animals by the injection of large amounts of normal nerve tissue.

(2). *The specific action of a "rabies toxin"* which is in combination with the fixed virus used for treatment.—Remlinger and Babes have held that this, together with an idiosyncrasy on the part of the patient, is responsible for the symptoms. Babes showed that animals injected with fixed virus which has been filtered or otherwise treated so as to eliminate infection by a micro-organism, do not die of rabies, but develop fever, emaciation, cachexia and paralysis and die after a protracted period. The theory of a toxin is a plausible one, but there is no consensus of opinion in regard to it.

(3). *Street virus infection,* received from the biting animal, and so modified by treatment that the disease runs an atypical and usually mild course, ending in recovery.

This view was held by Jos. Koch[32]. He inoculated animals from the lumbar cord of a fatal case. Some of these, after a long incubation, died of rabies. He explained the negative results obtained by Babes in four cases by the fact that Babes had used only the brain and medulla for inoculation, whereas it is the cord in these cases that is infective.

More lately Jochmann[38] has defended the same view, and has attempted to explain in accordance with this theory the cases, such as Franca's,[31] which seem to others to disprove it.

Against this view are the following facts: A number of cases have occurred in which the biting animal was not mad, and others in which the patients were not bitten, but had taken antirabic treatment merely as a precaution. Pain at the site of injury is rarely felt in paralysis cases. The incubation period is usually too short for infection with street virus. Paralytic rabies in animals is regarded as an intense rather than an attenuated form of the disease; therefore, street virus rabies, modified by treatment so as to become mild, should not be of the paralytic type.

(4). *Fixed virus infection due to the treatment.*—It is held by some writers that the unusually early appearance of symptoms in occasional cases is against this view. Strong emulsions are not commonly injected during the first days of treatment, yet the paralytic symptoms sometimes appear within seven days after the first injection of strong cord material. They also insist that if fixed virus possessed virulence for man, paralysis would be of frequent occurrence among the thousands of persons treated every year, whereas it is well known that it is very rarely seen.

Among others, Kozewalow[41] holds that fixed virus infection is responsible for most instances of paralysis incident to antirabic treatment. In defence of his position, he cites several fatal cases with necropsies followed by animal inoculations.

> (*a*) A case of his own with symptoms of ascending paralysis. No Negri bodies were found in the patient's brain. Animal tests comprised 33 rabbits, inoculated with the brain material. Of these, 24 died of rabies with an incubation of 6 to 7 days. The others died of sepsis.
>
> (*b*) Five cases from Bareggi[1] following treatment with fresh fixed virus. The patients died within a week of acute ascending paralysis. Rabbits inoculated from their brains showed symptoms of rabies in 5 to 6 days.
>
> (*c*) Franca's case, bitten by a dog not rabid (microscopic and inoculation tests negative). Patient developed ascending paralysis on the fifteenth day of treatment. She had received a one-day cord injection for the first time three days previously. On the 6th and 8th days of illness spinal fluid was withdrawn and injected into the anterior ocular chamber of three rabbits. After 11 days one of these died with symptoms of rabies. Two other rabbits were inoculated subdurally with the cord of this rabbit. Both developed rabies on the 7th day and died on the 9th day after vaccination. A rabbit injected intramuscularly with the cord of this rabbit died 9 days later with typical symptoms of rabies. The patient died on the 40th day of the illness.

It is said that this case has modified Koch's view so that he has admitted the possibility of fixed virus as well as street virus infection in these cases.

> (*d*) The case of Goldberg and Oszesalski[39]. A boy of 13 years, bitten by an unknown dog which was not captured. He received 6 injections in the next 4 days in a Warsaw institute. He then stopped treatment and walked 200 kilometers to his home. Thirteen days after the bite, and 12 days after the first injection, he was taken sick with symptoms resembling cerebro-spinal meningitis. The cerebrospinal fluid, however, was negative bacteriologically, and consciousness was retained throughout. The boy died suddenly from respiratory paralysis after 5 days. No Negri bodies found. The emulsified spinal cord produced rabies in rabbits in 7 to 8 days.

Kozenwalow[41] holds that the short incubation of rabies in the animals inoculated from the above mentioned cases, demonstrates that these paralyses are directly due to fixed virus infection. As further proof of his position, he states the following facts: At the beginning of 1909 the treatment in Cracow was intensified, and the use of a one-day cord begun, whereas, before that, nothing stronger than a two-day cord was used. Shortly afterwards they began to have cases of paralysis. Not less than 20 persons were affected in the course of a month. The symptoms were mild, and consisted of paresis of arm, or leg, or partial urine retention, and passed off in one or two days. The use of the one-day cord was then discontinued, and the symptoms stopped. He cites the experiments of Kraus and Fukahara[19], who proved that different strains of fixed virus in different institutes vary in virulence, since some are virulent subcutaneously in rabbits and white mice, while others are non-virulent. He gives the results of some experiments of his own, and states that each institute should analyze its own virus and determine whether it can or cannot use an intensive treatment.

In connection with these statements of Kozewalow, the experience of Poor and of the present writer are very suggestive. Of Poor's 12 cases, 1 occurred in 1906, 4 in 1907, and 7 during the nine months between February and October, 1908; after which, up to the time of his report, nine months later, no more cases had occurred. About October, 1908, he began to inoculate rabbits from the cord rather than from the brain of the rabbit of the preceding series. Thus he undoubtedly used a weaker virus, as the rabbits did not show initial symptoms until a day, sometimes two days later, than by the other method. He also gave smaller and weaker doses to those who were but slightly bitten.

The writer has used three different schemata of Pasteur treatment, the full course in each being 21 days.

The following table will show their comparative strength by indicating the number of injections of each cord in a full course of 21 days treatment. It also shows the number of cases of paralysis and of rabies which occurred among the patients treated under each scheme:

TABLE II

SCHEMES OF PASTEUR TREATMENT USED BY NEW YORK CITY DEPARTMENT OF HEALTH

Cord Dried	Scheme 1	Scheme 2	Scheme 3
	Jan. 1, 1912 to Aug. 2, 1913	Aug. 3, 1913 to May 6, 1914	May 7, 1914 to Present Time March 7, 1916
	Number of Injections in 21 Days		
Ten days	3	0	0
Nine days	3	0	0
Eight days	1	1	2
Seven days	1	1	2
Six days	1	1	2
Five days	1	2	2
Four days	5	5	9
Three days	5	7	8
Two days	5	8	0
Number of patients treated	1,408	811	911
Cases of paralysis	1	6 (one fatal)	0
Cases of Rabies	4	2	4

Thus it will be seen that Scheme 2 is somewhat stronger than Scheme 1, since it contains three more injections of two-day cord; and much stronger than Scheme 3, which includes nothing more active than three-day cords. Scheme 2 is very similar to the one employed by the Hygienic Laboratory of the United States Public Health Service, Washington, D. C., and by most of those who produce antirabic vaccine in this country, with the following differences: (1) The amount of cord per dose was only two-thirds as much as that employed in the Washington scheme. (2) We used a two-day cord instead of one-day cord on the 8th and 21st days of treatment.

One of the writer's cases of paralyses, Case 1, occurred in 1912 while Scheme 1 was in use. Other cases may have occurred under this plan previous to 1912, but the records were not available for examination.

Five of the remaining six cases, including the one terminating fatally, occurred between August 15th and October 17th, 1913, in the early weeks of the employment of Scheme 2. Although the plan was not changed, another case did not develop until the following spring. Strangely enough, in November and December, 1913, the rabbits used for producing the antirabic vaccine showed a tendency to develop initial symptoms in five or even four, instead of six days, and it was necessary to use lumbar cord and sometimes cord dried one day, for inoculation in order to bring the incubation back to six days. Still no paralysis cases occurred during this period. Finally on May 1st, 1914, at a time when the rabbits were coming down in exactly 6 days, another case of paralysis developed (case 7, a severe one, with recovery).

Scheme 3 was then adopted. In this no two-day cords are used except occasionally in a case of very severe face bite. Since then no instances of paralysis have occurred. (May 7th, 1914 to March 7th, 1916, a period of 22 months).

In the writer's one fatal case of paralysis the brain and cord were unfortunately put into formalin, so that no opportunity of trying animal inoculation was afforded. He therefore can express no opinion, based on experience, upon the important question whether these paralyses are due to fixed virus infection. However, there is no doubt in his mind that in most of the cases at least, the symptoms are caused, not by street virus infection modified by the treatment, but by the treatment itself either through the medium of fixed virus infection or through the action of a toxin, or both. In any event there must be a special idiosyncrasy on the part of the patient. Otherwise the cases would be enormously more frequent than they are.

Conclusions

1. Paralysis complicating the Pasteur antirabic treatment is very rare, but doubtless occurs in mild form more often than is known. It affects adults chiefly, young children almost never.

2. The prognosis is good in cases of mild or moderate degree, but the mortality among the severe cases is high.

3. The administration of antirabic treatment is therefore not entirely devoid of risk.

4. The possibility of the occurrence of paralysis in any particular case, however, is so slight that it should not deter those who evidently need antirabic treatment. A person bitten by a rabid animal is much more likely to develop rabies, which is certainly fatal, than paralysis, which usually ends in recovery.

5. The slight risk of paralysis should always be explained to those who are inclined to insist upon antirabic treatment in the absence of clinical or laboratory evidence that the biting animal was rabid.

6. In most instances this paralysis is caused by the antirabic treatment itself (fixed virus infection, or toxin, or both). It is not often due to street virus infection modified by the treatment. Its incubation is shorter than that of most cases of street virus rabies.

7. Variations in the strength of the fixed virus used are probably a factor in the etiology.

8. Personal idiosyncrasy is an important predisposing condition.

The thanks of the writer are extended to Dr. Anna W. Williams, Miss Caroline R. Gurley and Miss Alice G. Mann for making the laboratory studies in Case 6; to Dr. George B. Dunlap for his pathological report on the same case, and to the attending physicians, pathologists and house staffs of the Presbyterian, Fordham and Brooklyn hospitals for the privilege of studying the cases under their care.

Reports of Cases

The cases here reported comprise two groups:

A. Seven personal cases, treated with Pasteur Antirabic Vaccine supplied by the Department of Health, City of New York.

B. Six additional cases treated with Pasteur Antirabic Vaccine from another source.

A. The Writer's Personal Cases:

Case 1. L. S., male, 43 yrs.

Jan. 5, 1912.—Bitten by a dog; three punctured wounds on right thigh through trousers and drawers. Clothing not torn. Dog killed and Negri bodies found in brain.

Jan. 9.—Pasteur treatment was begun and continued for 17 days at the Research Laboratory, New York City, Department of Health.

Jan. 28.—Three days after completion of treatment, patient complained of numbness in both legs from knees down; also muscular weakness. Symptoms continued for several weeks.

Feb. 7, 1914.—Patient reported by letter that he still had muscular weakness of lower limbs, and an aching belt about one foot wide around the body including abdomen, back and hips. Had been able to work ever since the time of his treatment.

Case 2. E. H. C., age 52 yrs.; alcoholic history.

Aug. 1, 1913.—Bitten severely on hand by a dog which was killed and proven rabid by finding Negri bodies in his brain. (Dog bit five persons, all treated by same physician, to whom Pasteur treatment was sent by mail. The other four patients developed no untoward symptoms.)

Aug. 3.—Treatment was started.

Aug. 15.—The thirteenth day of treatment, patient began to have malaise, headache and weakness of limbs from thighs down.

Aug. 18.—Report by physician: has been in bed three days, temperature 101 degrees Fahrenheit or over, above symptoms more marked. Fell down in trying to walk from bed to chair. Paralysis not complete, reflexes normal. Retention of urine. Catheterized specimen showed no cystitis. No injections given on 18th or 19th.

Aug. 20.—Condition improved. Temperature normal, limbs not so weak. Electrical tests showed no reaction of degeneration. Pasteur treatment resumed.

Aug. 25.—Treatment completed. Has had twenty doses in twenty-four days. Is walking about, limbs still weak; he continues to require catheterization.

Nov. 2, 1913.—Letter from his physician states: tingling in anterior thighs, lower legs, hand (he has only one arm), and right shoulder started when he began to walk, and remains, through slight, up to the present time. He is now unable to run, but walks well, though he fatigues easily. Otherwise is in good condition.

Case 3. Mrs. G. H. E., 45 yrs., marked neurotic history. Not bitten, but licked on hands by her pet dog. Had a burn on one wrist and a small pustule on one hand. Dog had dumb rabies. Killed Aug. 10, 1913. Negri bodies found in the brain. Treatment, mailed to her physician, was begun Aug. 11.

Aug. 26.—After fifteen days of treatment, the following symptoms appeared: pain in head, back of neck and lumbar region, numbness in the hands and legs with some loss of power in legs, reflex and tactile sense normal; dysphagia, voice not natural. Antirabic treatment discontinued and not resumed.

Aug. 27.—Patient brought to the city and placed in Presbyterian Hospital where she was seen by the writer. Pain in head, back and limbs; rectal temperature, 99½ F. Upper and lower limbs very weak; could not lift herself to a sitting posture without assistance. When helped to her feet, could hardly stand. Reflexes normal. Marked weakness of tongue. Choked on attempting to swallow water. This was evidently due to paresis of muscles of deglutition, as there was no spasm and no dread of water. Examination of throat showed weakness but no paralysis of pharynx or larynx. Voice thick and husky. Patient said she slept poorly. She was nervous but not excited and did not think she had rabies. She was constipated but passed urine readily.

Aug. 29.—Slight improvement. Could speak and swallow better, but yesterday had such difficulty that the house physician was on the point of feeding her by gavage. To-day she can walk but totters. Pains continue.

Sept. 1.—Some further improvement. Temp. normal, no leucocytosis, speech and swallowing much better. Muscles of arms, back and lower limbs still weak but she could walk better.

Late in Sept.—Still in hospital but much improved.

Oct. 23.—Report by her physician. Is about well but muscles somewhat weak.

Nov. 29, 1913.—A letter from the patient says, "I have not recovered full muscular control but am progressing slowly."

Case 4. W. J. P. B., 37 yrs. About five weeks before treatment was started, he was bitten on the right thumb by a sick-looking stray puppy, which also bit his sister and brother-in-law. Dog killed, brain not examined. The brother-in-law died of rabies Sept. 8, 1913, and Negri bodies were found in his brain.

Sept. 9. Antirabic treatment was begun on Mr. B. and his sister. Mailed to the City Hospital, Newark, N. J. The sister had no ill effects at any time.

Sept. 27.—After the 18th injection, Mr. B. developed paralysis of left side of face, some ptosis and slight involvement of tongue. Three days later, other side of face became involved but less severely. Treatment was not interrupted. Complete recovery occurred after a few weeks.

CASE 5. Mrs. M. F., 32 yrs., bitten on one finger Sept. 30th, 1913, by a dog which soon afterwards died, presenting symptoms suspicious of rabies. Examination of smears from the brain failed to show the presence of definite Negri bodies.

Oct. 25.—Antirabic treatment was begun at Research Lab., Dep't of Health, under the writer's direction.

Oct. 17, after thirteen injections, she began to feel tired in the afternoon. In the evening while out for a walk she became dizzy and had a "queer feeling in the head." She reached home with difficulty, having fallen or fainted twice. Slept well but was restless in sleep.

Oct. 18.—She came to the laboratory and was seen by the writer. She complained that her lower limbs from ankles to hips, and her upper limbs from fingers to shoulders "felt as if asleep." She walked unsteadily. The dizziness and queer feeling in the head persisted. No Negri bodies had been found in the dog's brain, and inasmuch as the guinea pigs inoculated subdurally on October 6th, were still alive and well, antirabic treatment was discontinued and not resumed.

Oct. 20.—The patient's husband reported that she felt much better and could walk with ease. No further report was obtained.

CASE 6. G. E., 39 yrs., bitten Oct. 3, 1913. Six superficial tooth marks on right wrist. Dog killed, brain examined and Negri bodies found.

Oct. 5.—Antirabic treatment was begun at Research Lab., Dep't of Health, under the writer's direction.

Oct. 18.—He came to the laboratory with the following complaints; yesterday, after thirteenth injection, he began to have tingling and numbness in his lower limbs but worked all day. In the evening he noticed that he could not evacuate his bladder, succeeding only in dribbling a little urine. This morning, upon rising, he felt the same tingling and numbness from his feet to his hips. His limbs were so weak that he staggered about the room. With great difficulty, he passed about four ounces of urine. He went to work but soon had to stop on account of weakness in his limbs and he had great trouble in getting to the laboratory. Examination by the writer showed a weak, staggering gait, exaggerated patellar reflexes and marked distension of the bladder, with inability to pass any urine.

He admitted moderate alcoholic habit but denied syphilis or previous nervous disease.

This patient and the one reported above, Case 4, began treatment on the same day, developed paralytic symptoms on the same day and were seen by the writer at the clinic at the same time. It was a disconcerting situation.

Antirabic treatment was discontinued and not resumed. The man was urged to go to a hospital, but declined and insisted upon going home. His physician was telephoned to and advised as to his care.

Oct. 19.—In the evening, his physician reported that the patient had grown rapidly worse and was now completely paralyzed from the waist down. He was removed that evening to Fordham Hospital.

Oct. 21.—He was seen by the writer at the hospital. At that time there was complete motor paralysis and nearly complete anesthesia and analgesia of the entire body below a line drawn through the nipples, with no power to evacuate the bladder or rectum. Evidently there was marked paresis of the entire intestinal tract, as the abdomen was greatly distended and no flatus could be passed. There was also weakness but not paralysis of the muscles of the forearms and hands. Temp. 99 degrees to 100.5

degrees F. Resp. 20 to 30. Pulse 90 to 130. Occasional vomiting. No spasms or paralysis of throat muscles. Could swallow fluids and solids readily. Mind clear. No nervous excitement or apprehension.

Oct. 23.—Seen again. Instead of showing improvement, as the former cases had done, all symptoms persisted and the patient was generally weaker. Kidney elimination much diminished. Catheterized urine contained albumen, casts and red blood cells. From this time on until the end, his condition was pitiable. He lay in bed inertly, unable to move any portion of his body except his arms and head. The enormous distension of the intestines could be but slightly relieved by high enemata and rectal tubes. The weakness of his hands and arms increased but never advanced to complete paralysis. The voice became weak but no true throat paralysis developed. He had no difficulty in swallowing at any time. His vitality gradually waned and he finally died of respiratory paralysis on the evening of Oct. 28, eleven days after the onset of symptoms.

This case was variously diagnosticated by the physicians of the hospital as paralytic rabies, transverse myelitis and acute ascending paralysis of the Landry type.

Necropsy, Oct. 28, 9 P. M. The only gross lesions found were some hemorrhage into the intestinal canal and a spinal pachymeningitis. Portions of the cerebral cortex, hippocampus major, cerebellar cortex, and of the spinal cord at its cervical and lumbar enlargements and mid-dorsal regions were removed and set aside in their fresh state, some to be studied in the hospital laboratory and others to be sent the next day to the Research Lab., Dep't of Health, for examination with the special object of determining, if possible, the much mooted question whether animals inoculated subdurally from any or all of these parts of the cerebro-spinal axis would develop rabies, and if so whether the incubation of the disease in them would be long (two to four weeks), showing a street virus infection, modified clinically by the Pasteur treatment, or short (six to eight days), indicating a fixed virus infection due to the treatment itself.

Most unfortunately, an over-conscientious attendant, finding the specimens the next morning, thought the pathologist had forgotten to put them into a preserving fluid, and destroyed their usefulness for inoculation purposes by placing them in 10 per cent. formalin. Thus a great opportunity for valuable research was lost.

Since the brain and cord could not be examined for Negri bodies by the fresh smear method, they were hardened and examined in section but Negri bodies were not found.

Some of our material was very kindly examined by Dr. Geo. B. Dunlap, Pathologist of the N. Y. State Psychiatric Institute, Wards Island. Grateful acknowledgments are due him for permission to publish his report and to reproduce some of his microphotographs.

Pathological Report by Dr. George B. Dunlap

The material received consisted of blocks of tissue from the right cerebral hemisphere, including F_1, F_3, anterior central, posterior central, T_1, and the occipital region; also small pieces from the cervical, dorsal and lumbar regions of the spinal cord. In the cord specimens, especially in those from the cervical region, there was considerable injury and distortion caused by crushing. The blocks of cerebral tissue showed no special gross features. Samples were taken from these blocks for paraffin imbedding; the sections of the spinal cord, which were small, were imbedded as a whole. The stains used were chiefly hæmatoxylin and eosin, and toluidin blue; Weigert's myelin sheath stain was used on one section from the cervical cord.

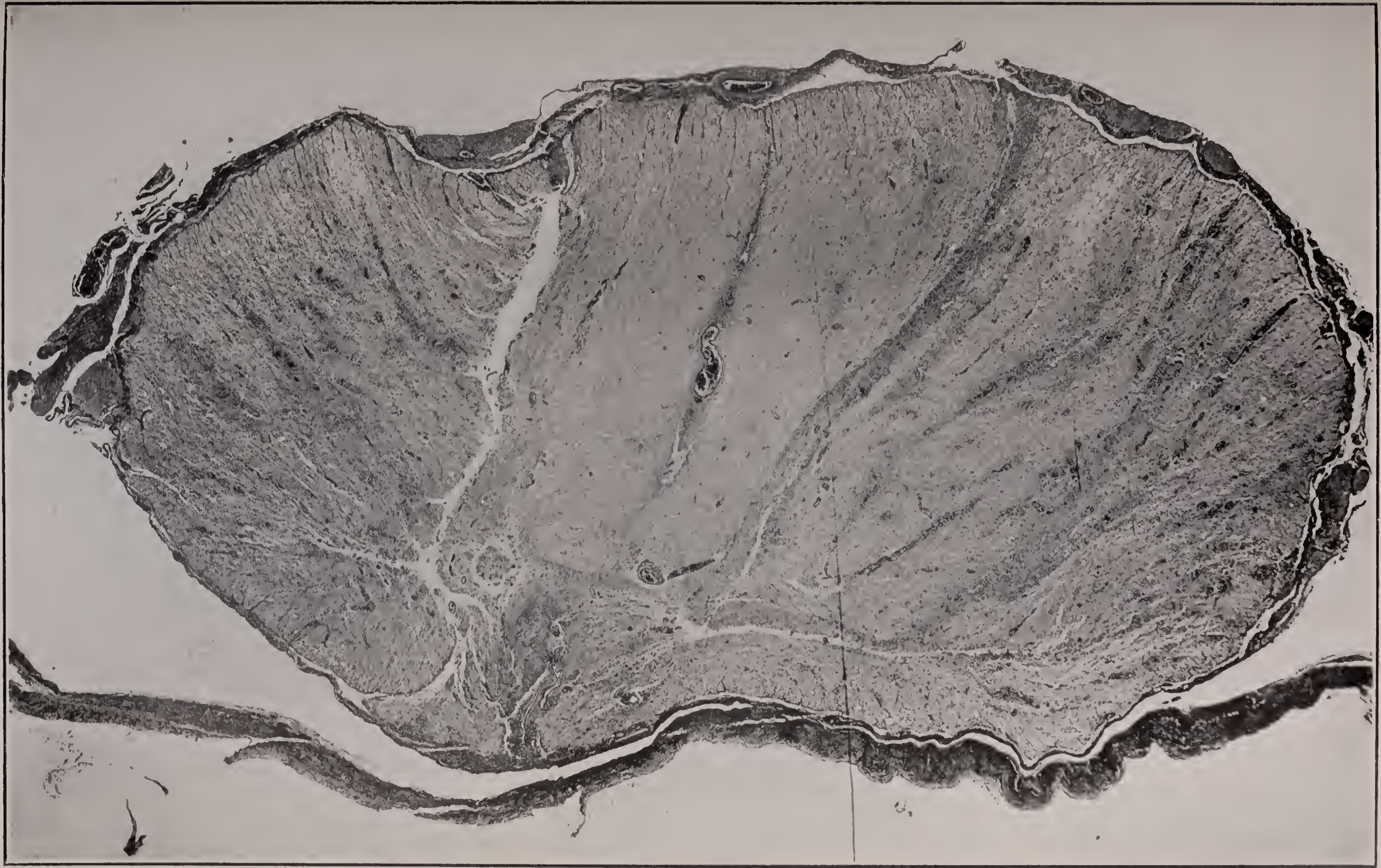
Microscopical Examination—(Summary)

Cortex—No changes indicating any definite disease process, with which the writer is familiar, were found. The Betz cells were in very fair condition; they contained a moderate amount of pigment; the smaller nerve cells were also in fair condition. In some sections, especially in the right anterior central cortex, satellite cells were rather numerous around the nerve cells of the deeper layers, but along the blood vessels they were not abundant. In the white matter of the brain the nuclei of the neuroglia, instead of being round, were often of irregular or elongated shape, but no other evidence of neuroglia reaction was noted. Some thickening of small blood vessels was generally noted in the pia covering the cortex, in the cortex itself, and in the sections of the spinal cord. In the blood vessels of the cord fibrous changes of fairly marked grade were not uncommon.

Spinal Cord—Sections from the three regions showed the same essential changes varying only in degree; namely, an acute stage of softening involving the fiber systems. This softening appeared first along either side of the various connective tissue septa of the posterior or lateral columns, and spread to involve parts more remote from the septa, and consequently more remote from the larger blood vessels which lie in these septa. The softenings were most extensive in the cervical segments, but were quite marked in the dorsal region where they varied in position in the different sections. They were least extensive in the lumbar segments.

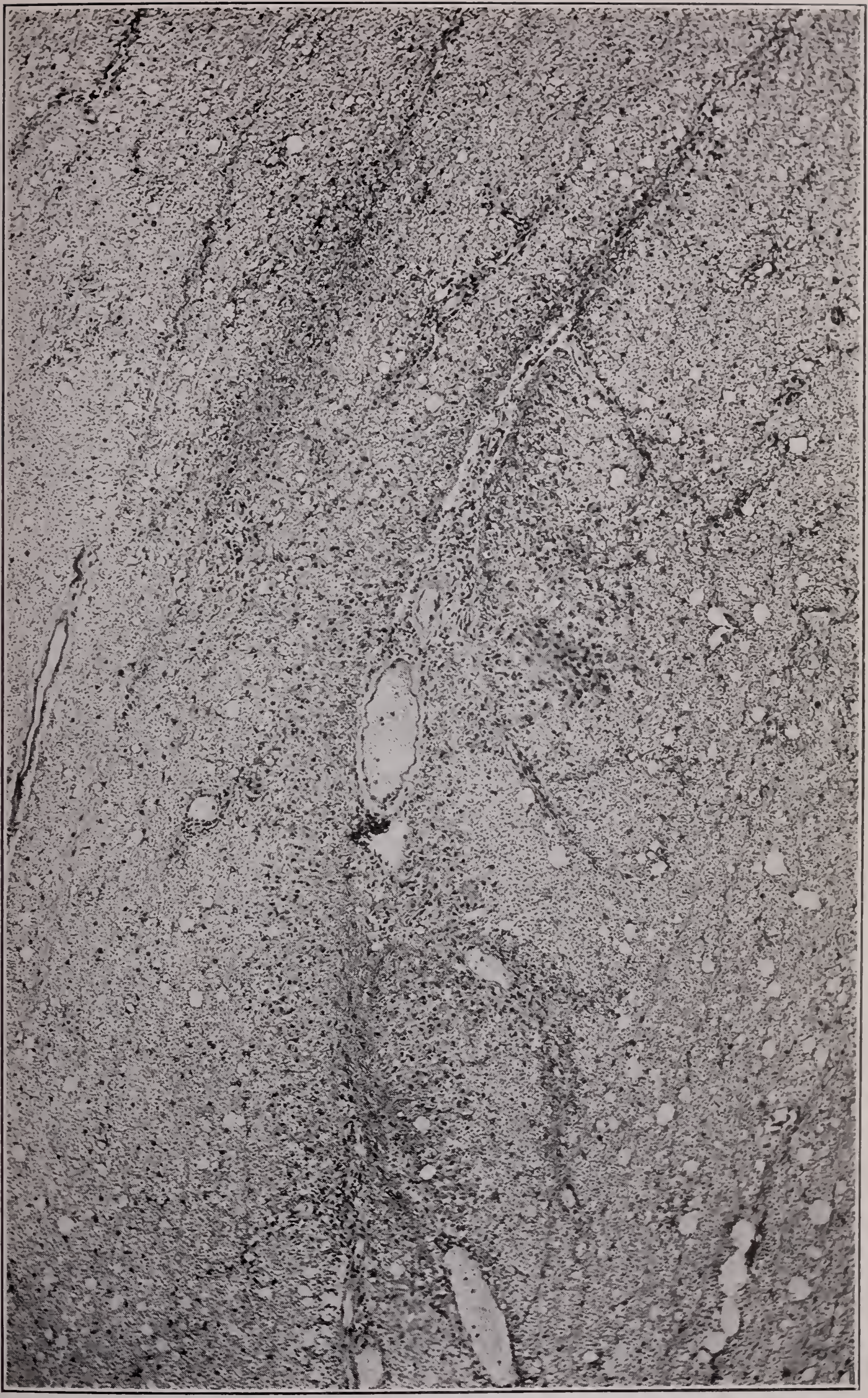
The softenings showed as more darkly stained areas or streaks in which the normal structures of nerve fiber and sheath had disappeared, and various types of cells and fragments of necrotic tissue were recognizable. The process in most places was too fresh to present large aggregations of plain granule cells (or " Gitter " cells of the Germans), but in most places scattered cells of this type were plainly visible, and were often loaded with fluid contents and debris (see photos). About many of the blood vessels in the softened areas were small round cells, lymphoid cells, which stained deeply; such cells might also be seen at a distance from the vessels; they seemed to be capable of enlarging and of taking up the products of the softening. Neuroglia fibers in the neighborhood of softenings were often abundant and coarse (a condition not well understood, unless explainable by swelling and heightened stainability of pre-existing fibers). The Weigert stained sections from the cervical cord, except for slight breaking down due to the softening were normal. The sections stained with toluidin blue were examined for bacteria, but none were positively identified. The writer has not seen changes, such as are present in this case, explained by the presence of bacteria.

Remarks—The writer fully realizes the highly speculative character of most histological interpretation and wishes this to be considered applicable to the following remarks. The histological condition of acute softening suggests the action of a strongly toxic material operating over a wide area in the spinal cord, and probably but not certainly delivered by the blood stream; the location of the earliest visible changes along the course of the blood vessels is not considered convincing proof of such delivery. In postpartum and various other conditions (infections?) similar changes in the spinal cord are sometimes found. Without any experience as to what it is possible for the virus of rabies to do in causing spinal lesions, the writer would still feel that this case with its definite pathological changes would speak rather for the introduction through the treatment of some toxic material in a susceptible individual than for a modified form of rabies, especially in view of the early appearance of symptoms thirteen days after treatment was begun and the duration of the process eleven days.



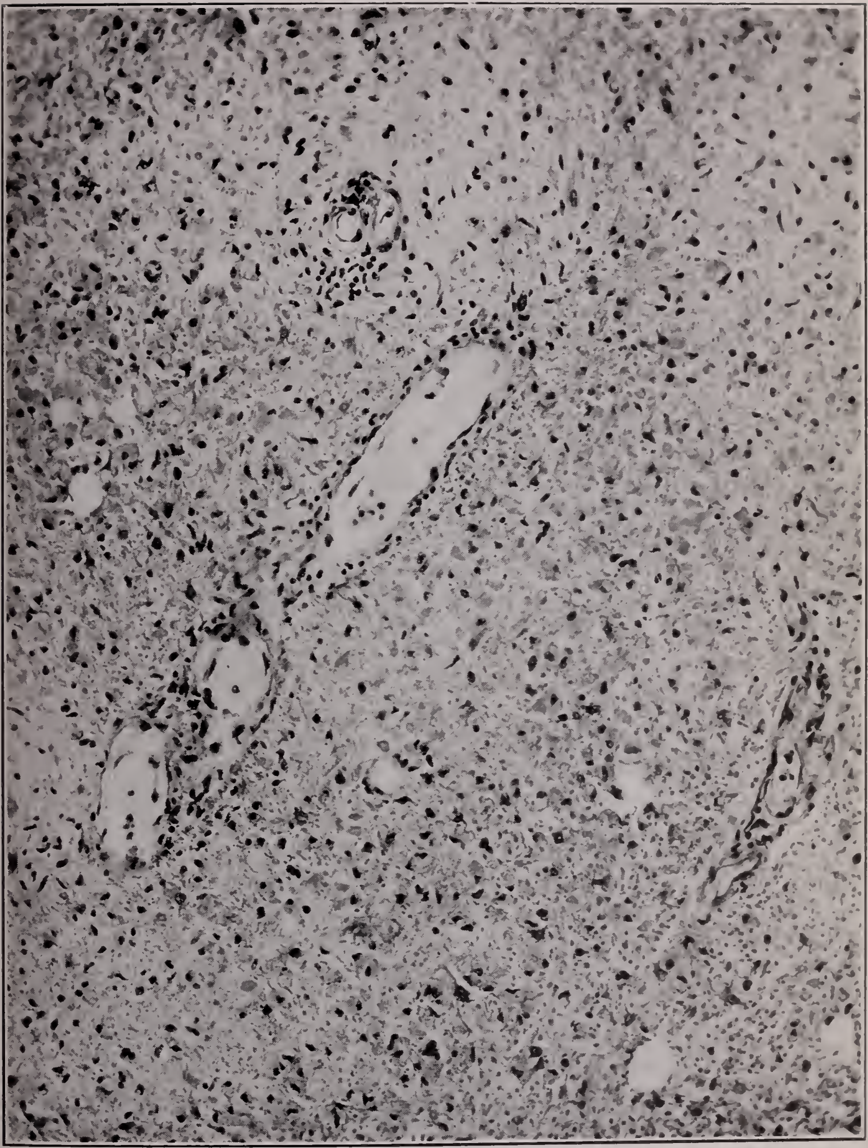
No. 824. Photo 1. Spinal Cord.

Cervical cord; considerably crushed; showing dark streaks of fresh softening, which follow in a general way the lines of the connective tissue septa. Right lateral column (of the photo) more swollen than the left.



No. 824. Photo 2. Spinal Cord.

Acute softening; showing as darker staining polycellular strips on either side of the connective tissue septa. The rest of the section, except for numerous small holes, looks practically normal, and shows plain axis cylinders surrounded by their sheaths.



No. 824. Photo 3. Spinal Cord.

A softened area, more highly magnified than in Photo 2; showing, very poorly, the Gitter or granule cells; these are best seen perhaps in the right lower quadrant. In the left upper corner of the photo axis cylinders and myelin sheaths which still retain their form may be seen. Note lymphoid cells in the sheaths of the blood vessels.

CASE 7. R. S., male, Italian, 35 years.—Speaks almost no English.

April 12, 1913.—Bitten between left thumb and index finger by one of three dogs which were together in a yard. He did not know which dog bit him. Three days afterwards he heard that one of the animals had been killed and sent for examination, but its carcass never reached the laboratory, and no history of the animal could be obtained.

April 17.—He came to the laboratory and insisted upon receiving the Pasteur treatment, as he was very much afraid of rabies. Treatment was started at the laboratory and continued at the Manhattan Antirabic Clinic, Department of Health.

May 1.—On the fifteenth day of treatment he complained of pain in the back like that of lumbago.

May 3.—Pain more severe in back, and running down the backs of the thighs.

May 4.—Patient went to the clinic, suffering acutely from sacral and sciatic pain. He had some loss of power in the limbs and required assistance to walk. Reflexes normal. No loss of control of sphincters. This condition was reported by telephone to the writer and recognized as a case of paralysis. Treatment was discontinued and was not afterwards resumed.

May 5.—Ill at home. His physician reported paralysis of both legs. Sphincters as yet unaffected.

May 6.—Taken to the Brooklyn Hospital. He then stated that his first symptom had been pain in the cardiac region, which soon passed off, and that on May 4th he had another attack of pain in the same region, more severe than the first, with a feeling of numbness affecting the whole left side below the level of the nipple. The numbness grew more marked and the left lower extremity became increasingly painful until he could not move it on that account. The right lower extremity became similarly affected but to a less degree. On admission he could not empty his bladder or rectum. Previous to the present attack he had always been in perfect health.

May 8.—Examination by Dr. Tilney, attending neurologist of the hospital. Patellar reflexes exaggerated. Achilles and Babinski reflexes and ankle clonus present. Paraparesis of both legs, more marked in left. Some hypertonus in both legs. Marked hypesthesia and hypalgesia in both lower limbs and in sub-umbilical zone. Numbness in both lower limbs and lower abdomen. A distinct girdle zone of hyperalgesia about four inches wide just above umbilicus. The patient complained of much pain here and a sensation as if a band were tied about his waist. Mind clear, but patient was mentally depressed. Neurologist's diagnosis: transverse myelitis; no prognosis ventured.

May 9.—Abdomen distended, enemas necessary. All urine is drawn by catheter. Wassermann negative.

May 13.—First signs of improvement. Patient could move left leg slightly, and raise knee about six inches from bed.

May 14.—Cerebro-spinal fluid not under pressure—clear. Examination negative. (Fluid inoculated subdurally into guinea pigs did not produce rabies.)

May 15 to 20.—Gradual improvement.

May 21.—Pain sense returned.

May 23.—First voluntary bowel movement. After the 26th, patient required neither cathartics nor enemata.

May 24.—Voided urine voluntarily for the first time since admission. No further catheterization required.

May 26.—Out of bed in chair.
June 2.—Able to stand without help but cannot walk.
June 4.—Walked a little without assistance.
June 9.—Walked home. Limbs still weak.
Six months later.—Patient's recovery practically complete.

B. Additional Cases:

The following six cases of paralysis occurring among 1143 patients treated are reported in brief from the records of a pharmaceutical firm which distributes the Pasteur antirabic treatment quite widely through the United States. So far as the writer knows they have not hitherto been reported.

All the patients were adult males—all recovered. All the biting animals were rabid.

1.—Bitten April 3, 1913. Treatment was begun April 30, finished May 25.

May 27, twenty-three days after beginning treatment, and two days after its completion, patient developed right facial paralysis. Duration, four weeks.

2.—Contaminated with saliva of rabid cow. Treatment was begun June 4, discontinued June 17.

June 15, the twelfth day of treatment, paralysis began in lower limbs, and in three days involved body from waist down, including sphincters. Improvement began June 24. Reported as well Dec. 9, 1913.

3.—Bitten June 16, 1913. Treatment was begun July 8; finished July 28.

August 7, thirty-one days after beginning, and ten days after completion of treatment, patient developed right facial paralysis. Duration, two weeks.

4.—Bitten Nov. 7, 1913. Treatment was begun Nov. 21; continued with some interruptions for twenty-four days, and finished Dec. 14.

Dec. 5, sixteen days after beginning of treatment, first symptoms appeared, pain in legs which continued throughout his illness.

Dec. 10. Slight facial paralysis. Dec. 11, legs weak, pain less.

Two months later, paralysis had disappeared, still weak and easily tired. After six months, still somewhat nervous and more easily fatigued then formerly.

5.—Bitten March 10, 1915. Treatment was begun March 16; finished April 4.

March 29, the fourteenth day of treatment, symptoms began with pain in bladder followed by paralysis of that organ and of left leg.

Ten days later, condition not good. No further report.

6.—Bitten March 14, 1915. Treatment was begun March 19; discontinued March 31.

March 30, the twelfth day of treatment, had retention of urine with pain and tenderness in legs. Sent to hospital eight days later. Symptoms still persisted. No later report.

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THREE CASES OF GONOCOCCUS SEPTICEMA WITH ARTHRITIS FOLLOWING SCARLET FEVER

Matthias Nicoll, Jr.

(*Reprinted from Archives of Pediatrics, November,* 1914)

The following 3 cases of generalized gonococcus infection with arthritis seem worthy of record, not on account of the rarity of the condition, but as demonstrating the necessity for routine blood cultures in cases of so-called scarlatinal arthritis. The patients were admitted to the scarlet fever hospital of the Department of Health in the service of Dr. Alfred Hess and that of the writer.

Case I. Mollie S., aged twenty-one months, admitted April 24, 1914. The physical examination was negative except for the presence of desquamation and a congested pharynx. As there was some vaginal discharge, she was sent to the ward for "observation" vaginitis cases, although an examination of a number of smears of the vaginal discharge, made at various times during her stay in the hospital, failed to show the presence of Gram-negative cocci.

The temperature during the first ten days did not rise above 100°.

On May 6th there was a sudden rise of temperature to 107°, and, as the appearance of the throat suggested diphtheria, 10,000 units of antitoxin were given, following which the temperature fell rapidly to normal, but rose again almost to as high a point two days later.

May 11th, a blood culture showed the presence of gonococci. There was a septic rash on the body, an otitis media on the right side, and an arthritis of the right wrist. The lungs, heart, abdomen and throat were negative.

May 13th, arthritis of left wrist. Ulcerations about the mouth with profuse oral discharge. Fifty million polyvalent gonococcus vaccines were given.

May 14th, 100 c.c. of polyvalent gonococcus serum given subcutaneously. Neither this nor the vaccines had more than a temporary beneficial effect on the temperature and pulse, which showed great daily excursions.

May 18th, 50 c.c of gonococcus serum given.

May 22d, there was a systolic murmur heard most intensely over the pulmonic area. The swelling of the right wrist was less marked.

June 13th, blood culture negative for gonococci.

The administration of vaccines was continued on every third or fourth day, the dose being increased by 50,000,000 at each injection until a maximum of 600,000,000 was reached, on July 7th. Following the earlier injections there was usually a temporary rise of temperature, but in the last three weeks they produced no apparent effect on the temperature, which, except for a few slight exacerbations, did not rise much above 100° up to the time of the patient's discharge, on July 12th. On leaving the hospital the involved joints showed a moderate amount of thickening with some limitation of function, but no visible enlargement. The patient had lost a good deal of weight and was very anemic.

August 4th, a visit paid to the patient's home showed an improvement in her general condition. The right wrist was slightly tender, the left wrist weak. There was no ankylosis in either. There was a soft, blowing systolic murmur at the apex of the heart, transmitted to the left and heard at the lower angle of the scapula. The pulmonic second sound was accentuated. The heart was somewhat enlarged.

In an attempt to determine the portal of entry of the blood infection, in addition to the several vaginal smears, material from the tonsils and ulcerations about the mouth were also examined for Gram-negative cocci, with negative results.

Case II. Lazar N., aged five years, was admitted to the hospital April 28th, ill one day with an attack of scarlet fever of moderate severity. The temperature on admission was 102.5°, pulse 140, both falling to normal on the fourth day. On physical examination there was noted a faint systolic murmur at the apex of the heart.

May 4th, there was a rise of temperature to 104°, the cause of which could not be ascertained.

May 12th, there was a papulo-erythematous eruption on the legs and arms, which on the following day became pustular in a few of the lesions. The right wrist was very tender, the temperature rising to nearly 107°.

May 14th, a blood culture showed the presence of gonococci.

May 15th, the eruption had spread slightly over the chest and abdomen.

May 16th, 50,000,000 polyvalent gonococcus vaccines given, the temperature at the time being 105°.

May 19th, the temperature and pulse had fallen almost to normal; the right and left wrists were swollen and tender. The systolic murmur at the apex was transmitted to the axilla.

June 1st, wrist joints much improved; left elbow involved.

June 2d, blood cultures negative for gonococci.

June 3d, all joints much improved.

June 19th patient was discharged in fairly good condition.

A visit made to the patient's home, on August 5th, showed that the general condition had improved since leaving the hospital. All the joints were normal. There was a systolic murmur heard at the base of the heart, probably of hemic origin.

It may be remarked that there had never been at any time any evidence of urethritis in this patient; nevertheless it is unfortunate that urethral smears were not examined for gonococci. The writer has reported a case of general gonococcus infection in a male child in which gonococci were found to be present in the urethra, although there had been absolutely no evidence of urethritis.

Case III. Celia S., aged six and one-half years, admitted to the hospital on June 8th, ill four days with scarlet fever of rather a severe type. Physical examination showed a heavy scarlatiniform rash over the entire body. The throat was markedly congested, with exudate on the tonsils. There were moist râles over the lung bases, posteriorly. Temperature 105°, pulse 170. The vaginal smears were suspicious of gonococcus infection, and on June 26th gonococci were found.

June 12th, blood culture showed the presence of gonococci.

June 13th, the patient's condition had been grave up to this date, when the temperature and pulse fell to normal. A slough in the pharynx had involved the deep tissues and caused perforation of the anterior pillars of the fauces.

June 14th, there was a general maculo-papular eruption over the body; the ankle joints were swollen and tender. The temprature rose to 103½°.

July 3d, a blood culture was negative for gonococci. The patient made a gradual recovery under purely symptomatic treatment, no serum or vaccines being used, and was discharged on July 28th in good condition.

The occurrence of so-called scarlatinal arthritis is a fairly common complication of scarlet fever in hospital practice, its frequency varying in different epidemics. The causative factor, notwithstanding numerous theories in regard to it, remains as little known as that of scarlatinal nephritis, although the occurrence of both during the convalescent stage of the primary disease would suggest a common origin. Blood cultures taken from cases of scarlatinal arthritis of the ordinary type are usually negative, nor do the course of the temperature and general symptoms of most of the cases suggest the presence of general sepsis.

In the tendency toward chronicity, and the production of more or less thickening and limitation of function in the involved joints, the disease resembles more closely gonococcus arthritis than ordinary acute articular rheumatism, and furthermore differs from the latter in that it frequently occurs in children under five years of age, while, on the other hand, the liability to endocardial involvement seems to be equally pronounced in both.

How many cases of scarlatinal rheumatism, occurring especially in hospital practice, are really due to gonococcus infection it is not possible to say, but there can be no doubt that a number of such cases escape recognition through neglect to take blood cultures.

The presence of the eruption not usually seen in cases of scarlatinal arthritis, suggesting the presence of some blood infection, was largely responsible for the taking of blood cultures in the 3 cases reported and thus led to a correct diagnosis.

The prognosis of gonococcus infection of the blood in children, while grave, would seem to be less so than in adults and the liability to destructive heart lesions less pronounced. The value of gonococcus vaccines in the more or less chronic complications of gonorrhea would lead one to expect benefit to follow their use in cases of arthritis. It cannot be said, however, that the course of the disease in the two cases in which they were here used differed radically from that of the one treated symptomatically.

The writer wishes to express his thanks to Dr. Hess for permission to publish an account of these cases.

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PURULENT CONJUNCTIVITIS IN INFANTS UNDER TWO MONTHS OF AGE

Incidence, Causes and Effect on Vision

Anna Wessels Williams and Caroline Rosenberg

The investigations reported in this paper were undertaken by the Bureau of Laboratories of the Health Department, incited chiefly by the Committee on the Prevention of Blindness of the Sage Foundation, in order to help determine the present incidence, causes and results of purulent conjunctivitis in infants under two months of age in New York City.

We are calling the condition investigated *purulent conjunctivitis of infants* rather than ophthalmia neonatorum, because even yet, notwithstanding the admirable work of Stephenson and others calling attention to the fact that several micro-organisms besides the gonococcus might produce this condition, the majority of physicians consider ophthalmia neonatorum and gonococcus infection of the conjunctiva in the newborn as synonymous.

We thought that if we could bring out further statistics to show that this opinion is incorrect, it might help do away with the feeling of reluctance to report such cases to the Health Department.

Incidentally, we wished to find out how many cases of this type were not reported to the Health Department.

We desired further to determine the number of cases in which preventive treatment was not used and the effect on the course of the cases.

The investigations are still incomplete, but we thought the first report might be given at this time.

Incidence of Purulent Conjunctivitis in Infants

The question of the incidence is being studied by securing data in relation to the condition of the eyes of 1,000 consecutive births in two definite districts, one in the poorer part of the city, the other in the more well-to-do. The first district chosen is between 14th St. south to the River, and between 3rd Ave. and East River. The investigation in this district has just been completed. The children born on the 1st of December, 1914, and successively thereafter have been visited. 42 cases were not seen, because of removal or wrong addresses. Hence, the 1,000 cases reported here are among the first 1,042 births.

In our record card we notice date of birth, name and address of obstetrician, date of onset, character of symptoms and treatment. Where and by whom treated, possible source of infection, present condition of eyes.

15 babies died, 2 of whom gave history of purulent conjunctivitis, 6 children, still living, had purulent conjunctivitis, two of which were probably due to infection with the gonococcus. All were treated and have eyes normal now. 14 children were seen who had conjunctivitis with no history of purulent discharge and had normal eyes at time of visit. 978 cases, then, in the thousand, had normal conjunctivas.

Only one out of the eight cases was reported to the Health Department and this was a midwife case. One of the others was a midwife case, the other six belonged to physicians.

These statistics may be arranged in tabular form as follows:

Condition	Cases	Percentage
History of normal eyes	978	97.8
History of purulent conjunctivitis	8	.8
History of conjunctivitis without pus	14	1.4

Causes of Purulent Conjunctivitis in Infants

Data and cultures were obtained, made from a series of cases reported to the Health Department chiefly by the midwife service.

From November 17, 1914, to July 31, 1915, 91 cases in all were reported to the Health Department; 43 were found to be either treated by a physician (17 cases), or were incorrectly reported, the eyes being normal. There remain 48 cases from whom data in relation to signs, onset, possible prophylactic treatment, possible source of infection, were secured and from 47 of which cultures were taken.

For the study of the micro-organisms in the eyes of infants having purulent conjunctivitis, the following is our method of procedure:

1. (a) Note is made of the appearance of the eyes; (b) whether a discharge be present or not; if present, the character and amount of it; (c) the condition of the eyelids, whether edematous or inflamed or encrusted; (d) the condition of the conjunctiva lining the lids, and of that covering the eye-ball; (e) the condition of the cornea.
2. The conjunctivae lining the lids of the eyes are gently curetted. Smears are made from one currettment, and as soon as partially dried by the air, are fixed in methyl alcohol. The material from the next currettment is placed in tubes of blood-broth. The next is placed in a small amount of ascitic fluid. Each eye is then washed with boric acid.
3. The mother is advised as to what should be done and referred to the nearest eye dispensary.
4. The smears are stained by the Giemsa method and by Gram.
5. Blood-drop agar plates and plates of acid agar (Sabouraud's medium) are inoculated by a loopful of material from the blood-broth.
6. From the ascitic fluid small amounts are placed in tubes of kidney ascitic fluid. Different dilutions are made. Each is covered by a layer of sterilized petroleum and incubated anaerobically. These are examined on about the 4th, the 10th and the 15th days. Smears are made and stained by Giemsa method—Stab inoculations are made in deep, narrow tubes of semi-solid ascitic agar. Aerobic and anaerobic plates are made.

From the original blood broth and from the blood-drop agar, transplants on other media, and smears are made until identification of the different organisms is established.

The acid agar medium is used for determining the presence of molds and yeasts.

By our method of using enriching media for the curetted material we are reasonably sure that if living gonococci are present we can detect them.

Organisms found. The following table shows the incidence of the bacteria found in the 47 cases of purulent conjunctivitis from which cultures were made:

Microorganism	In Number of Cases	Percentage
Staphylococcus	47	100
Xerosis bacillus	42	89.3
Pneumococcus-streptococcus group	22	46.3
Gonococcus	12	25.5
Influenza bacillus group	12	25.5
Morax-Axenfeld bacillus	3	6.3
Sarcinae	3	6.3
Bacillus subtilis	1	2.1

In two cases only—4.2%—were inclusion ("trachoma inclusion") bodies found. In one case—2.1%—indefinite bodies which somewhat resembled "trachoma inclusion" bodies were seen.

Staphylococcus and *B. xerosis* were commonly found, but usually in small numbers. Only in one-fourth of the cases was the gonococcus definitely found to act as an etiological factor. 20 cases where no gonococci were found have been considered as of possible gonococcus origin because of early date of onset, and time elapsing between onset and date of first visit. These twenty cases all made a good recovery.

The pneumococcus-streptococcus group were demonstrated about twice as often as were the gonococcus and the B. influenzae group, and two last-mentioned groups of organisms, were found in an equal number of times in this series of cases. In relation to the Streptococcus-pneumococcus group, the first three cases in which they occurred, no record was kept of their type, but from February 4th to April 6th, including the next six cases in which they appeared, they were of the haemolytic type; while the following 13 cases from April 6th to date, were of the viridens types of infection.

In the anaerobic cultures no micro-organism was found which had not appeared in the aerobic cultures.

1. *Date of Onset.* The *ages* of the infants at the time of the first visit, which was usually within 24 hours of the reporting of the case, were from 3 to 47 days.

11 were seen during 1st week
23 were seen during 2d week
8 were seen during 3d week
4 were seen during 4th week
1 was seen during 5th week
1 was seen during 7th week

The *onset* of the disease took place as follows:

4 or 8.3% at birth
28 or 58.3% during first 3 days
35 or 72.9% during first week
10 or 20.8% during second week.
3 or 6.3% unknown (as they were " foundlings " brought to the Manhattan Eye and Ear Hospital)

2. The *onset* of the cases where the following bacteria were found was as follows:

Gonococcus, from birth to the 14th day	1 at birth 4 during 1st week. 2 during 2d week..	= 6 cases
Streptococcus-pneumococcus group from birth to 10th day	1 at birth 11 during 1st week 1 during 2d week..	=13 cases
Influenza group from 2d to 11th day	3 during 1st week. 2 during 2d week..	= 5 cases
Gonococcus and *Streptococcus-pneumococcus groups*	1 on 2d day = 1 during 1st week 1 on 13th day = 1 during 2d week	= 2 cases
Influenza and *Streptococcus-pneumococcus groups* from birth to 9th day	2 at birth 5 during 1st week.. 1 during 2d week..	= 8 cases

The *gonococcus,* the *influenza* and the *streptococcus-pneumococcus groups* only 1, which occurred on the 7th day or during the 1st week. The remaining 10 cases of *staphylococcus* and *xerosis bacillus* infection from 3d to 13th day, 8 during 1st week and 2 during 2nd week.

In 37 cases or 77% it was determined through mothers' statements that a prophylactic was used. Mothers' statements, however, cannot be accepted as reliable. In all probability there was a higher percentage than 77% which had received prophylaxis.

After Treatment. In 13 cases there had been no treatment up to time of seeing infant. In 34 cases boric acid had been used by mother, and among these, where infant had been in hospital or to dispensary, argyrol also had been used. Three cases mother used breast milk, thinking it was beneficial.

With the exception of one midwife, who reported cases 37 and 38, there was not more than one case from the same midwife.

Possible Source of Infection. It was impossible to elicit sufficient history of the families and of those who had come in contact with the infants, to get sufficient data to point toward a probable source of infection in each of the cases. The following eleven cases were the only ones in which possible associative infection could be found.

In case 13, where influenza bacilli were found, onset 8th day, 2 other children had coryza and bronchitis.

In case 19, where influenza bacilli were found, several other children of family had "colds."

In case 26, where a streptococcus culture was obtained, nurse, with whom baby slept, had "cold."

In case 27, where influenza bacilli were found, mother had "cold."

In case 28, where influenza bacilli were found, child died from "cough." He had had a "cold" soon after birth.

In case 29, where streptococci were found, another child had nasal discharge and "cold."

In case 31, where streptococci were found, mother had "cold."

In case 32, where streptococci and influenza bacilli were found, mother and another child had "cold."

In case 35, where streptococci and influenza bacilli were found, another child in family had "cold."

In case 45, where gonococcus was found (onset 4th day), mother had a profuse vaginal discharge. Another child, now 2½ years, with eyes now normal, had had purulent conjunctivitis.

DEFECTIVE VISION RESULTING FROM OPHTHALMIA NEONATORUM

In determining the relationship of defective vision to purulent conjunctivitis, we have noted (1) the organisms found in these cases, (2) the date of onset, (3) the relation of onset of the use of prophylactic and curative treatment, and (4) the questions of source and complication of infection.

So far we have had only three cases in which vision is probably impaired through corneal opacities, one less than the other two. Gonococci, staphylococci and xerosis bacilli were the only organisms found in these cases. In all prophylactic treatment was used. The onset of one was on the fourth day, of the second on the thirteenth day, and of the third on the first day. In the first and third the source of infection was not determined, in the second the mother had clinical gonorrhoea.

SUMMARY AND CONCLUSIONS

(1) In only 0.8 per cent. of a thousand consecutive births in a given district did purulent conjunctivitis occur.

(2) In 10 of 47 cultured cases, or in 21.2%, only staphylococcus or staphylococcus and the Xerosis bacillus were found, but not in large enough numbers to be considered a cause.

(3) In 12 of the 47 cases, or in 25.5% the gonococcus was found. In 9 of these 12 cases, or in 19.1% only of all the cases, was the gonococcus found without the presence of either the streptococcus-pneumococcus group or the influenza group of organisms. In 3 of these 12 cases, or 6.3% of all the cases, the gonococcus was associated with either or both the streptococcus-pneumococcus and the influenza groups.

(4) In 22 cases, or in 46.3% of all the cultured cases, was the streptococcus-pneumococcus group of organisms found independent of the presence of the gonococcus and the influenza bacillus. It was thus found to be apparently about twice as often a cause of purulent conjunctivitis as the gonococcus.

(5) In 12 cases, or in 25.5% (the same number of times as gonorrhoeal infection), was the influenza bacillus group found as the apparent exciting cause.

(6) In three cases only was the cornea involved. The fact that these cases were gonorrhoeal infections confirms the opinion that cases of purulent conjunctivitis which are infected with this organism are liable to be more severe in infants than those due to other infections.

(7) A history of 77% of cases positively receiving prophylactic treatment, and of a probably greater percentage, having received it, suggests the advisability of treatment on the second day with a $Ag\ NO_3$ solution as a prophylactic against secondary infections.

(8) Purulent conjunctivitis in infants is due in one-third of the cases at least to organisms other than the gonococcus.

(9) The fact that purulent conjunctivitis or ophthalmia neonatorum does not always mean an infection with the gonococcus, should make physicians more ready to report such cases to the Health Department.

THE SCHICK REACTION AND ITS APPLICATION

William H. Park and Abraham Zingher

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The definite association between the presence of antitoxin in the blood and immunity to diphtheria has been firmly established by the efficiency of passive immunizing doses of antitoxin in protecting individuals against diphtheria, and also by finding a complete absence of antitoxin in those who develop clinical diphtheria.

The presence of natural immunity to diphtheria in a large number of people has become a well established clinical fact—this immunity is generally associated with the presence of antitoxin, as shown by finding it in measurable quantities in the blood of the majority of such individuals. A small percentage of individuals seem to be protected by bactericidal rather than antitoxic substances. The determination of the presence of antitoxin by the older methods requires much time and the use of guinea pigs. Schick's merit is that he devised a simple clinical test[1] by which we can easily determine the presence of antitoxic immunity.

The reaction depends on the local irritant action of minute quantities of diphtheria toxin when injected intracutaneously. If antitoxin is absent in the cutaneous lymph, or present only in very small amounts, insufficient for neutralization, a positive reaction will appear in twenty-four to forty-eight hours. This is characterized by a circumscribed area of redness and slight infiltration which measures from 1 to 2 cm. in diameter. It persists for seven to ten days, and on fading, shows superficial scaling and a persistent brownish pigmentation. The amount of toxin injected as advised by Schick is 1/50 MLD. for the guinea pig in .1 c. c. of normal saline. We prefer 1/50 MLD. in .2 c. c. on account of fairly severe local reactions seen occasionally with the greater concentration of the toxin in individuals in whom not even a trace of antitoxin is present.

It is important to distinguish the true reaction from a pseudo-reaction which is found very rarely in young children, but in a larger percentage of older children and especially in adults, who have antitoxin in either a small or large amount. These reactions are probably local sensitization phenomena of a protein character, since similar reactions can be obtained almost uniformly in these persons with dilutions of an autolysate of the Klebs-Loeffler bacillus which is free of toxin, with toxin heated to 75° C. for 5 minutes, or with overneutralized diphtheria toxin. In a few cases, the beef broth alone, less diluted however, will give the same result. The pseudo-reaction can be distinguished clinically in most cases from the true reaction. It appears earlier, is more infiltrated in some of the cases, less sharply circumscribed and disappears usually in 48 to 72 hours. It leaves only a faintly pigmented area, which, in our experience, never shows superficial scaling. In the rare cases in which the nature of the reaction remains in doubt, it is best to consider the reaction as positive.

For the carrying out of the test, it is essential to have an accurate syringe with a sharp, but short-pointed, fine needle. The usual 1 c. c. "Record" tuberculin syringe with a fine platinum-iridium needle answers the purpose well. A standard ripened diphtheria toxin is diluted at first 1 : 10 in sterile physiological salt solution; this dilution will keep in the ice-box with little deterioration for at least two weeks. For use, further fresh dilutions are made in normal saline, of such strength that .2 c. c. contains 1/50 MLD. for the guinea pig. This amount is injected intracutaneously on the flexor surface of the arm or forearm. The rather persistent pigmentation may make the use of the forearm objectionable; in such cases the surface of the arm may be chosen.

A simple outfit[2] for the Schick test is now supplied by the Bureau of Laboratories to physicians in New York City. It consists of a capillary tube that contains a little over one M.L.D. for the guinea-pig, of an undiluted, ripened diphtheria toxin, a small rubber bulb for expelling the toxin, similar to the ones used with vaccine outfits, and a bottle containing 10 c. c. of sterile normal saline. When the toxin is added to the saline, a dilution is obtained, of which 0.2 c. c. represents 1/50 MLD., the amount used in the test. (See paper—Methods of using diphtheria toxin in the Schick test, and of controlling the reaction, p. 284.)

Though the intensity of the reaction varies in different individuals, a well-marked redness indicates an almost complete absence of antitoxin. Faint reactions point to the presence of very small amounts of antitoxin, which are not sufficient, however, to certainly protect the indvidual against diphtheria, but do prevent the constitutional poisoning. To prevent the appearance of the reaction, according to Schick, at least 1/30 unit of antitoxin per c. c. of blood is required. This amount he considers sufficient to protect against diphtheria. According to v. Behring, even as little as 1/100 unit of antitoxin will protect against the disease in uncomplicated cases.

The Schick reaction was carried out[3] during the past 18 months on all patients entering the Scarlet Fever Pavilion of the Willard Parker Hospital. In all, 1,200 patients were thus tested, and of these, 656 or 54.7 per cent. gave negative reactions. Only cases giving positive reactions were immunized; those giving a negative reaction received no immunization, but were carefully observed. Although about 20 per cent. of the negatively reacting patients became bacillus carriers during their stay in the wards, no cases of clinical diphtheria developed among them.

The remaining 544 patients, who gave positive reactions, received in practically all cases some form of active immunization. Among those who reacted insufficiently to active immunization with toxin-antitoxin mixtures or vaccines made from Klebs-Loeffler Bacillus, 72 developed clinical diphtheria. The majority of the cases were of a mild tonsillar or anterior nasal type.

According to age, the number of reactions obtaned were as follows:

SCHICK REACTIONS AMONG SCARLET FEVER PATIENTS

Age	Total	−Schick	+Schick	%+Schick
6 mos. to 1 year	10	5	5	50.0
1 to 2 years	68	22	46	67.6
2 to 4 years	223	93	180	65.9
4 to 6 years	249	116	133	53.4
6 to 8 years	196	123	73	37.2
8 to 15 years	237	171	66	27.8
Over 15 years	167	126	41	24.5
	1,200	656	544	45.3

Several groups of healthy persons were also tested with the following results:

SUMMARY OF SCHICK TESTS FROM 3 LARGE ORPHAN ASYLUMS (NORMAL CHILDREN)

Age	Total	−Schick	+Schick	%+Schick
2 to 4 years	62	42	20	32.2
4 to 6 years	318	236	82	25.7
6 to 8 years	444	347	97	21.8
8 to 10 years	597	462	135	22.6
10 to 12 years	584	459	125	21.4
12 to 14 years	506	416	90	17.7
14 to 16 years	189	158	31	16.4
	2,700	2,120	580	21.4

SCHICK'S RESULTS

Age	Total	−Schick	+Schick	%+Schick
Newborn	291	275	16	7
First year	42	24	18	43
2 to 5 years	150	55	95	63
5 to 15 years	264	133	131	50
	747	487	140	34.9

The percentage of individuals susceptible to diphtheria is greatest, therefore, between the ages of one to four years. It is less during the second six months of life and less in older children and in adults. These results in cases of scarlet fever closely approach those obtained by Schick, whose table shows positive reactions in 7 per cent. of the newborn, in 43 per cent. during the first year of life, in 63 per cent. between two and five years and 50 per cent. between five and fifteen years. In adults the positive reactions were not more than 10 per cent. It is rather important to note in our table of normal children, that not more than 17 to 32 per cent. of children between the ages of 2 and 14 years gave a positive reaction. This is a significant factor in the active immunization against diphtheria with mixtures of toxin-antitoxin.

In an interesting study of the relationship of the antitoxin content in the blood of the mother and new-born v. Gröer and Kassowitz found that 84 per cent. of mothers and their new-born show a considerable amount of natural antitoxin. The antitoxin of the infant is evidently derived from the mother through the placenta and because of its frequency it is regarded as a physiological phenomenon. The mothers also showed a pseudo-reaction in 47.5 per cent. of cases. This is an unusually high percentage and the toxic culture fluid must have been exceptionally rich in extract of the bacillus substance. Similar reactions could be obtained in these mothers with the same toxic culture fluid after it was neutralized with diphtheria antitoxin. In 11 per cent. of the new-born they obtained a negative Schick reaction, even though the blood examination revealed an entire absence of antitoxin. This they considered as due to the fact that the reaction on part of the new-born to toxin did not show itself by the usual clinical signs.

During systematic testing of groups of children according to families, we were impressed with the frequency which all the children of the same family gave a similar reaction. If variations were found, the younger children always gave the positive reactions. If the youngest child had a negative reaction, all the older children were usually negative. On the other hand, if the oldest child in a family gave a positive reaction, the younger children with very few exceptions showed positive reactions. These striking facts are additional proofs that there are factors, possibly hereditary in character, which in the absence of infections with the Klebs-Loeffler bacillus (v. Behring Kleinschmidt) give rise to the presence of the so-called natural antitoxin. The large amount of antitoxin which is present in some of the cases is hard to explain; for example, two young children, six and one-half and three years of age, who had no history of clinical diphtheria, showed respectively fifteen and nineteen units of natural antitoxin per c. c. of serum. The large proportion of older children and adult persons having antitoxin adds to the difficulty of considering the natural antitoxin as usually due to a previous infection with diphtheria bacilli either as a case or as a carrier. In a recent investigation published by v. Gröer and Kassowitz the combining and physical characteristics of the antitoxin present in persons who had never had diphtheria appears to be identical with that due to immunization.

With this test Schick attempted to place on a more rational experimental basis some interesting clinical problems in connection with the prophylactic and therapeutic dosage and mode of administration of antitoxin. By making the test at regular intervals previous to the injection of antitoxin, he found that neutralization of toxin in contact with tissue cells was still possible up to a certain number of hours. This was shown by the partial or complete suppression of the reaction. The efficiency of the injected antitoxin depended on the mode of administration and the size of the dose.

RELATIVE EFFICIENCY OF DIPHTHERIA ANTITOXIN ADMINISTERED IN DIFFERENT WAYS

Name	Age	Units	Admin.	20 Hours Previous	6 Hours Previous	4 Hours Previous	2 Hours Previous	Simul.	2 Hours Subsequent
A. N.	6^1	1,000	S. C.	....	+++	+++	++	±	–
J. R.	4^9	1,000	I. M.	....	+++	+++	+	–	–
I. W.	5^6	1,000	I. V.	....	+	±	–	–	–
W. S.	5	20,000	S. C.	....	±	±	–	–	–
A. F.	6^6	10,000	I. V.	++	....	....	....	....	....
G. S.	6	10,000	I. M.	+++	....	....	....	....	....
F. M.	6^2	10,000	S. C.	+++	....	....	....	....	....

We carried out some parallel experiments (see table) attempting at the same time to see approximately how much more effective the same dose of antitoxin was when given intravenously than when given subcutaneously or intramuscularly. The Schick test was made six hours, four hours and two hours before, at the time of the injection of the antitoxin and 2 hours later. With 1,000 units given intravenously the Schick reaction from the toxin injected six hours previously was considerably weakened though not entirely suppressed. The reaction from the test made four hours before appeared very faint, while that made two hours before was negative. 1,000 units given subcutaneously had no effect on the toxin injected four and six hours previously, and only weakened the reaction made by the toxin given two hours before, while that from the simultaneous injection was just visible. The same amount (1,000 units) given intramuscularly had a somewhat stronger effect than the subcutaneous injection, and prevented the appearance of a reaction from the simultaneous injection. The action of the toxin injected in the test 2 hours after the administration of 1,000 units of antitoxin was completely inhibited.

To obtain the same effect with the subcutaneous as was obtained with the intravenous injection of antitoxin, from ten to twenty times the amount was needed; 20,000 units given subcutaneously were able to affect the reaction due to the toxin given six hours previously to the same extent as 1,000 units given intravenously. When the Schick test was made 20 hours before the injection of the antitoxin, the intravenous injection of even 10,000 units of antitoxin had only a very slight effect in lessening the reaction, while the subcutaneous and intramuscular injection of the same amount of antitoxin had no effect whatever upon the reaction. In very toxic forms of diphtheria, therefore, where a large amount of toxin has already come in contact with the cells of the body with a resulting greater or less amount of fixation, large doses of antitoxin (10,000-20,000 units) should be given intraxenously to neutralize as much as possible the effect of such partially-fixed or contact toxin. No further injections will then be necessary.

The Schick reaction was also used to determine the duration of passive immunity after the usual dose of 1,000 units of antitoxin. This was found to vary in the great majority of cases between 21-25 days, although occasionally we have obtained a positive reaction at the end of 15-18 days. This corresponds with clinical observations made on immunized persons. The effect of a previous injection of antitoxin upon the duration of passive immunity as given by a second dose of antitoxin can also be studied in a very interesting way by using the Schick reaction. According to v. Behring, a primary injection of 1,000 units of antitoxin will protect for 3-4 weeks, whereas a secondary injection of a similar amount given at a time when the body is still sensitized by the first injection, will protect for only 5-7 days. This increased destruction of antitoxin is attributed by v. Behring to the production in the body, as a result of the first injection of the antitoxic horse-serum, of a proteolytic ferment, which causes a more rapid breaking down of the second dose of antitoxin. Römer & Viereck and Lewis have shown the same increased destruction of antitoxin in sensitized animals.

Last year we had an opportunity of verifying these statements during an epidemic of diphtheria at a colored orphan asylum at King's Park, L. I. About 150 children, who gave positive Schick reactions, were immunized with 1,000 units of antitoxin. At the end of 30 days they were retested and a positive reaction again obtained. These children were reinjected with a second dose of 1,000 units of antitoxin, and we now found that fully 60 per cent. had destroyed the second dose in 7 days, and another 10 per cent. in 10 days. This halving of the duration of the second or third period of passive immunization as compared with the first is important to consider when a longer immunity is desired than that given by the first injection.

Kolmer and Moshage have recently claimed that in some cases of diphtheria there is a rapid destruction or neutralization of the antitoxin which had been given for therapeutic purposes. This apparent elimination was so rapid that they were able to obtain what they believed to be positive Schick reactions at the end of 5 days in fully 17 per cent. of patients who had received therapeutic doses of 10,000-100,000 units of antitoxin. This conclusion was entirely opposed to our own investigations. Since the authors made no accurate distinction between true and pseudo reactions, we assumed that their patients had antitoxin, but gave a pseudo reaction to the Schick test. To verify this assumption we tested 46 patients who had had clinical diphtheria, tonsilar or laryngeal, from 5-22 days previously, and had then received from 5,000-20,000 units of antitoxin. We obtained 6 pseudo reactions and 40 negative reactions. The examination of the blood of the patients who had given the pseudo reaction showed the presents of from ½-2 units of antitoxin per c. c. in each case. A later paper by the same writers infers that they now think themselves that they were misled by the pseudo reactions. It is safe to assume that every case receiving 10,000 units of antitoxin has a considerable amount of antitoxin in the blood for a period of one week.

Nature of Immunity Developed After an Attack of Diphtheria

The application of the Schick test to patients that have had diphtheria some time previously, has revealed some interesting points in connection with the type of immunity which they develop after the disease.

We attempted to trace a number of the patients who had had definite tonsillar exudates with positive cultures. Thirty-two such patients, who had been treated at the Willard Parker Hospital 3-4 months before, were tested by Dr. Rosenberg during an investigation of discharged contagious disease cases. Nineteen of the 32 gave positive Schick reactions while 13 were negative.

Fifteen children were also tested at the Howard Colored Orphan Asylum, King's Park, L. I. Seven of the 15 had had diphtheria about one year before; of these 5 gave positive and 2 negative reactions; 8 cases had had the disease about 4 months ago, and of these 7 gave positive and one a negative Schick reaction.

At the Willard Parker Hospital four patients were tested who were suffering from a rather mild type of diphtheria; three tonsillar and one nasal. The Schick reactions were strongly positive on admission—no antitoxin was given, and the exudates cleared up at the end of 4-5 days. Tested with the Schick reaction two days after the disappearance of the exudate, it was again found strongly positive in every case. A similar strong reaction was obtained two and three weeks after the disease. These children had evidently developed little or no antitoxic immunity, and yet they made an uneventful recovery. Considering the small amount of antitoxin necssary to suppress the Schick reaction, we are forced to the conclusion that these patients had developed a bactericidal immunity only. The sera of these patients gave no complement fixation.

The chronic tube cases, however, who have been at the Willard Parker Hospital for more than a year, show, as a rule, a negative reaction. Patients who had diphtheria recenty (2-3 months), frequently give a positive reaction which becomes negative if they remain in the hospital for another 5-6 months. One child who had diphtheria in November, 1913, gave a positive reaction in January, 1914, and a negative reaction in August, 1914. Three other children who had diphtheria in March, 1914, were tested about the middle of August, 1914. One gave a negative, one a moderately positive, and one a strongly positive reaction. They were retested 2½ months later, and the following results were noted: The negative case remained negative; the patient that had given a moderately positive reaction showed now a very faint reaction, while the strongly positive case gave a much weaker reaction at this time. When we tested these children again two months later, e. g., eight months after the illness, they all gave a negative reaction, and blood examinations showed the presence of antitoxin.

The absence of antitoxic immunity in a large proportion of individuals who have had diphtheria, as seen at all tests in the children who received no therapeutic antitoxin injections and in tests made after the disappearance of the horse antitoxin in those receiving injections, leads us to assume that diphtheria patients develop as a rule an anti-bacterial immunity, which is associated in only about one-third of the cases with an antitoxic immunity. Chronic reinfections with the Klebs-Loeffler bacillus, as seen in children who remain a long time in the diphtheria wards, appear to finally lead in a majority of the cases to the production of an antitoxic immunity.

Results of Injection of Toxin-Antitoxin Mixture

The above results, obtained in children after an attack of diphtheria, are very significant in connection with the results which we obtained in the active immunization with mixtures of diphtheria toxin and antitoxin.

We carried out our work on 3 groups of children in the scarlet fever wards of the Willard Parker Hospital. In the early stage of the work, we soon noted that all individuals who had a natural antitoxic immunity, produced a very decided increase in the amount of antitoxin after injections of toxin-antitoxin. The response was very prompt and followed even after a single injection. These individuals were, however, naturally immune, and needed no further immunizing injections.

Entirely different results were obtained in the patients who had no natural antitoxic immunity. An early response (within 4 weeks after the injections) to the active immunization was noted in only 25 per cent. of the injected non-immune individuals. The amount of antitoxin produced in these patients was sufficient to completely suppress the Schick Test. About one-half of the remaining 75 per cent. gave fainter reactions than in the control tests obtained before the injections of toxin-antitoxin; thus showing the development of a small amount of antitoxin, which was not sufficient, however, to definitely protect these patients against diphtheria. When they were retested, however, from 6 weeks to 18 months after the immunizing injections, we found that fully 80-85 per cent. of those who had failed to give a sufficient early antitoxin production, were now immune, as shown by a negative Schick reaction. This late development of antitoxin is interesting and throws some light on one of the many factors that have to be considered in the active immunization of human beings. The same phenomenon has been noted in the immunization of guinea pigs with toxin-antitoxin. Our results show that active immunization is successful in human beings and is applicable where no immediate protection is required. In such individuals who are exposed to diphtheria, a combined active and passive immunization is advisable.

For our results in active immunization with toxin-antitoxin, see the following 2 papers:

(1) Active Immunization in Diphtheria and Treatment by Toxin-Antitoxin.

(2) Late Results in the Active Immunization with Diphtheria Toxin and Antitoxin, and with Toxin-Antitoxin Combined with Diphtheria Bacilli.

CONCLUSIONS

In reviewing, then, some of the more important applications of the Schick reaction we have to emphasize:

(1) The great reliability of the test, when the toxin and the method of employing it are correct, in showing the presence or absence of antitoxic immunity to diphtheria: a negative reaction obtained after the age of 3 years, indicates that the individual is protected, probably indefinitely, against the disease. The great majority of positive reactions in children are true reactions and indicate an absence of antitoxin, and therefore, unless other anti-bodies are present, a susceptibility to diphtheria. Among adults the pseudo-reaction is seen in a certain proportion of individuals, but this reaction can usually be distinguished clinically from the true reaction. The two reactions may be found combined in the same individual; in such cases of the psuedo-reaction will disappear in 3-4 days, leaving a distinct circumscribed area of scaling pigmentation due to the positive reaction. The use of some of the same lot of toxin heated to 75° C. for 5 minutes or after neutralization with antitoxin, as a control, will help to indicate the pseudo-reactions.

(2) The great value of the test in determining clinically the efficiency of the immunization of susceptible individuals, who have been injected with mixtures of diphtheria toxin and antitoxin. For this purpose only positive Schick cases should be chosen, and after the injections they should be tested at intervals of one, three, six and twelve months, to determine whether a sufficient amount of antitoxin had developed early or late to inhibit the Schick reaction, showing thereby the production of an active immunity to diphtheria.

(3) The Schick test is of great help in clearing up the diagnosis of clinically doubtful cases of diphtheria. With a purulent or sanious nasal discharge showing the Klebs-Loeffler bacillus, it is difficult to decide whether the case is a carrier or a beginning diphtheria. A negative reaction excludes diphtheria, while a positive Schick reaction leaves the diagnosis of diphtheria still a probability. A case of tonsillitis due to streptococcus in a carrier of diphtheria bacilli would, by the use of the culture alone, be thought to have diphtheria and in danger of extension of the disease. A negative Schick reaction would indicate the case to be simply a carrier, and in no danger from the effects of the diphtheria poison.

(4) The Schick reaction has added further proof to the clinical and experimental observations that very toxic cases of diphtheria do better when given an early intravenous injection of antitoxin than when it is administered in any other way. It reveals the fact that an intravenous injection of antitoxin is able to partly neutralize toxin six hours after its absorption by the tissues, and thus gives us hope in some of the late cases of diphtheria. The results with the Schick test will serve as a reminder that after contact with the tissues for a few hours the effect of the toxin can no longer be prevented; that a day, and, in fact hours of delay in the administration of a therapeutic dose of antitoxin may mean not only the absorption, but the final binding of a fatal dose of diphtheria toxin.

(5) The results obtained with the Schick test in families seem to indicate that besides infection with virulent diphtheria bacilli, other factors, possibly hereditary in nature, are concerned in the production of natural immunity to diphtheria. The absence of antitoxic immunity in more than 65 per cent. of individuals after an attack of diphtheria and in the majority of those soon after treatment with mixtures of toxin and antitoxin, if

they did not possess it before treatment, shows that the tissues have never had, or have never acquired, the ability to readily produce antitoxin.

(6) The Schick reaction can be applied with advantage in testing the patients, resident staff and nurses of contagious disease hospitals. By its use a considerable saving of antitoxin can be effected during outbreaks of diphtheria in institutions or in homes; it relieves of the fear of contagion and also avoids unnecessarily sensitizing about one-half of the exposed individuals. It should be applied as a routine in both the measles and the scarlet fever pavilions of hospitals. By administering 1,000-2,000 units of antitoxin in positive Schick cases and reimmunizing the patients if they remain for more than three weeks in the hospital, we should be able to control the development of diphtheria during the convalescence from scarlet fever or measles.

In conclusion, we wish to state that the ability now to recognize clinically those individuals who are susceptible to diphtheria, will arouse an interest in the active immunization with mixtures of diphtheria toxin-antitoxin which will enable us to control and possibly finally eradicate the disease. Up to the present time, in spite of the use of antitoxin, the number of cases of diphtheria has but slightly diminished. The brilliant lowering of the death rate has been due to the lessened mortality in the treated cases rather than to a decrease in the total number of cases of diphtheria.

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A SIMPLE OUTFIT FOR THE DISTRIBUTION OF DIPHTHERIA TOXIN FOR THE SCHICK TEST

Abraham Zingher

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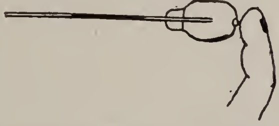
Owing to its reliability, the Schick test has found a constantly increasing field of application. The great difficulty in popularizing the test and placing it within the reach of the general practitioner and hospital worker has been, on the one hand, the inconvenience of handling the undiluted toxin, and, on the other hand, the rapidity with which the diluted toxin deteriorates on keeping, either in solution or tablet form. The outfit here described has been devised to furnish a standard amount of undiluted diphtheria toxin, which remains of fairly constant strength when kept cold in the ice-box, and can be readily diluted when required. We have every reason to believe that it will be found convenient and reliable.

The outfit for the Schick test consists of a capillary tube, which contains the toxin, a small rubber bulb, similar to the ones used in the distribution of vaccine virus, and a bottle containing 10 c.c. of sterile physiological salt solution. The capillary tube contains a little over one minimum lethal dose for the guinea-pig of undiluted diphtheria toxin. To allow for possible deterioration of the toxin during the following 6 months, a slight excess is added (1¼ M.L.D.). The exact amount of toxin is filled into each capillary glass tube by means of a 0.25 c.c. Record syringe and a fine needle. The toxin is then gently shaken toward the middle of the capillary tube, and the ends quickly sealed in the flame. If this is carefully done, the toxin will not be affected by the heat during the process of sealing the tube. The capillary glass tubes should have a medium bore, so that the toxin will fill about 1/3 to 1/2 of the length of the capillary. The toxin used for this purpose should not be a freshly prepared lot, but have ripened for at least a year, and its M.L.D. for a 250 gram guinea-pig carefully determined.



Method of Using Capillary Tube and Rubber Bulb

To use the outfit, one end of the capillary tube is broken off and carefully pushed through the neck of the rubber bulb, until it punctures the diaphragm within, and enters the cavity of the bulb; the other end of the tube is then broken off. The bulb is now held between thumb and middle finger, the index finger placed over the opening in the larger end of the bulb and the toxin expelled into the 10 c.c. of saline. The capillary tube is rinsed out by drawing up saline several times, the bottle is then corked and the diluted toxin shaken. 0.2 c.c. of the dilution representing 1/50 M.L.D., is injected intracutaneously on the flexor surface of the forearm or arm. The contents of the bottle are sufficient for about 35 tests. On account of the fairly rapid deterioration, it is not advisable to use the diluted toxin after 12-24 hours. The outfit must be kept very cold to prevent deterioration.

A 0.5 or 1.0 c.c. Record tuberculin syringe and a fine platinum-iridium needle are preferred. An ordinary hypodermic syringe may be used, but it should be graduated to 0.2 c.c.

Outfits with 6 and 12 capillaries are convenient for institutions, where a fresh solution has to be prepared daily. By refilling the 10 c.c. bottle with sterile salt solution or cool boiled water, a fresh dilution of toxin can be readily prepared.

Control outfits may also be put up similar to the ones used for the Schick test. Each capillary tube contains the same amount of toxin, which has been heated, however, to 75° C. for 5 minutes to destroy the soluble toxin. The control solution, which contains the protein of the autolyzed diphtheria bacilli, but no more free toxin, is used in testing for pseudo and combined reactions.

It is important to remember that, in using diphtheria toxin in the Schick test, we are dealing with an accurate quantitative reaction, and handling carefully measured amounts of an active agent, that has a tendency to deteriorate, even in bulk, if it is not properly protected from light and exposure, and kept in a very cold place.

AN ECONOMICAL INTRACUTANEOUS METHOD FOR TESTING THE VIRULENCE OF DIPHTHERIA BACILLI

Abraham Zingher and David Soletsky

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The testing of the virulence of isolated strains of diphtheria and diphtheria-like bacilli is important in particular for the following reasons:

1. Four to eight per cent. of individuals in localities where diphtheria is endemic are carriers of bacilli morphologically and culturally like the diphtheria bacillus, but non-virulent in 30–50 per cent. of cases. At the Willard Parker Hospital, Wilcox and Taylor found that 4.5 per cent. of the cases admitted to the scarlet fever wards were bacillus carriers, and of the isolated organisms only one-half were virulent.

2. According to Neisser, individuals who become persistent carriers after an attack of diphtheria show only non-virulent forms in fully 20 per cent. of cases. The absence of virulence in the organisms isolated from these carriers is probably permanent, since in our opinion these bacilli are not derived from the virulent bacilli which excited the disease, but rather from bacilli which were originally non-virulent. Therefore, if this fact were established, such individuals could be discharged from quarantine.

Morphologically, the non-virulent strains cannot be separated from the virulent, and in their sugar fermentation reactions the two types are in a majority of instances similar. Hence, the animal test must be employed for final diagnosis in doubtful cases. The method thus far in vogue for testing the virulence of diphtheria bacilli has been to isolate the strain, grow it for 48 hours in ascitic broth (1 part ascitic fluid + 2 parts veal broth), and inject 1 c.c. of the broth culture subcutaneously into a guinea-pig. A control pig is injected with the same amount of the broth culture, and also a small quantity of antitoxin (0.5 c.c of a 100 or 200 unit antitoxin, which cannot be used for other purposes.) If the strain is a true diphtheria organism, the control pig will live, while the test pig will die in from 2 to 3 days, and the autopsy will show the typical lesions of death from the effects of diphtheria toxin—subcutaneous, often extensive oedema at the site of injection, congested and hemorrhagic adrenals, fluid in the pleural cavities and congestion of the lungs with areas of partial consolidation.

This method is reliable, but an autopsy should be performed in all cases in order to exclude death from other causes.

Recently M. Neisser has suggested that the virulence of cultures be tested as follows: One loopful of a 24-hour Loeffler slant of the organism is suspended in each of 1 c.c., 10 c.c., and 100 c.c. of physiologic salt solution, and 0.1 c.c. of each suspension is injected intracutaneously into the abdominal surface of a guinea-pig. As a control, some antitoxin containing 8 units per c.c. is added to an equal volume of the heaviest suspension, and 0.1 c.c. of the mixture is injected intracutaneously into the same guinea-pig. True virulent diphtheria bacilli will cause a characteristic local inflammatory lesion, with superficial necrosis, in 48 to 72 hours, the intensity of the reaction depending upon the number of injected organisms and their virulence. The skin at the site of the control injection should remain normal in appearance. No lesions are produced when the Bacillus Xerosis or Bacillus Hoffmanni, or the non-virulent diphtheria-like bacillus is injected.

This method is analogous to that of Römer for the determination of small amounts of diphtheria antitoxin in sera. This consists of the intracutaneous injection of varying mixtures of the unknown antitoxic serum and a standard toxin; a slight excess of toxin produces a local necrosing lesion which is in every way similar to that produced by virulent diphtheria bacilli. while a neutral or over-neutralized mixture shows no effect on the tissues at the site of injection.

The method recommended by Neisser is fairly satisfactory, but, following his directions, we have occasionally noted that the direct addition of antitoxin to the bacteria in the control injection immunized the animals sufficently to affect the test lesions to a considerable degree. If the amount of antitoxin added is diminished to avoid this general immunization, the local action of the bacteria is not completely inhibited, and lesions are found in both test and control areas.

For this reason the following modification of Neisser's method, which has been found to be both reliable and economical, is suggested.

Method.—Two guinea-pigs, weighing about 350 grams, are used for the testing of from 4 to 6 different strains. One pig as a control receives 1.0 c.c. of antitoxin (about 500 units per c.c.) intracardially or intraperitoneally. The hair on the abdominal surface of each pig is removed, either by the application of a paste made of barium hydro-sulphide, or preferably by simply pulling the hair out. This can be easily done, with less pain, probably, than is associated with the prolonged irritation following at times the application of the depilatory.

For the bacterial emulsion, the entire surface growth from a fresh 24-hour culture from an ordinary Loeffler slant or a glucose ascitic agar slant of equal size is suspended uniformly in 20 c.c. of normal saline. It is important that the growth be not more than 24 hours old, since many of the bacteria die if the culture is kept for 48 hours or longer in the thermostat. Ice-box preservation of grown cultures for more than 2 days also kills many of the organisms. Loeffler slants are used similar to those furnished by the New York City Department of Health for the purpose of diphtheria diagnosis. They should have a fairly uniform surface size and be sealed with paraffin to prevent drying of the medium. It is also important that the medium be not too acid, since excess of acid is apt to inhibit growth to a considerable degree.

Suspensions of the cultures to be tested are prepared in the way described, and 0.2 c.c. of each is injected intracutaneously into corresponding areas of the abdominal surfaces of both pigs. The animals are securely fastened for this purpose to suitable holders. The abdominal surface is divided into 4 to 6 areas, depending upon the size of the guinea-pig, and the injections are made as far apart as possible in order to avoid a fusion of the lesions. Four strains can be tested out on a medium-sized guinea-pig (about 350 grams), and six on a larger one, without the danger of overlapping. A 0.5 c.c. or 1 c.c. Record syringe with a very fine steel (Burroughs, Wellcome & Co., No. 1) or platinum iridium needle (Burroughs, Wellcome & Co., No. 22) is suitable for the injection. If the injections have been made properly a circumscribed elevation appears, which persists for from 1 to 2 minutes.

The results of the tests are noted in 24, 48 and 72 hours. Virulent strains produce a definitely circumscribed local inflammatory lesion, which shows a superficial necrosis in 48 to 72 hours. In the control pig the skin remains normal if the injections have been accurately carried out. With non-virulent strains, no lesions will be found in either control or test animal.

In this way, four cultures can be tested on two animals, as compared

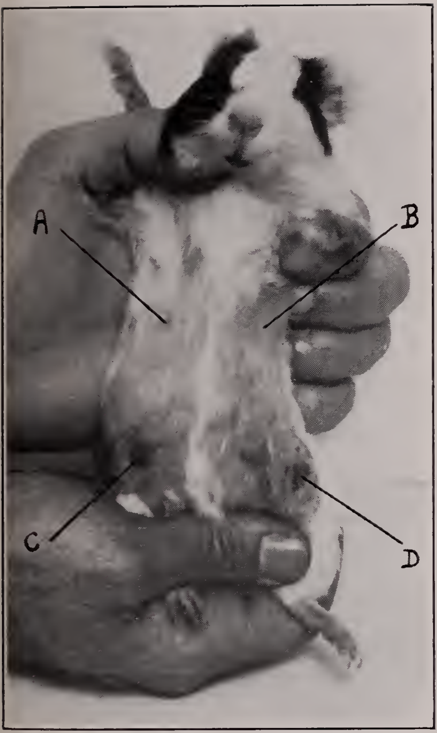


FIGURE I. TEST ANIMAL.

This guinea-pig received intracutaneous injections at points A, B, C and D of suspensions of four different strains of morphologically typical diphtheria-like bacilli. At A and B there are no lesions, an absence of virulence being thus indicated in these strains; at C and D there are distinct, circumscribed, indurated lesions with beginning superficial necrosis, showing that these two strains are virulent.

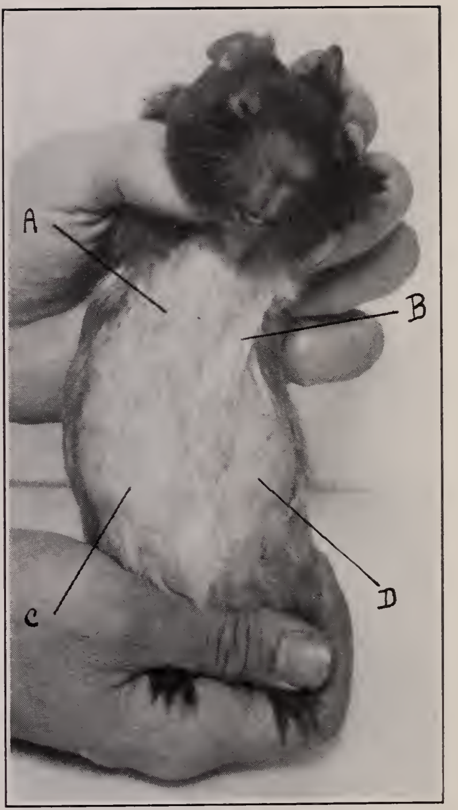


FIGURE II. CONTROL ANIMAL.

This animal received four injections corresponding to those received by the guinea-pig in Fig. I, and, in addition, 0.5 c.c. of antitoxin intracardially. No lesions were produced at C and D, hence, the specificity of the lesions shown in Fig. I is proved.

with eight animals necessary in the older way. The control animal may be used again within a week for another set of tests without a further injection of antitoxin. By the use of large pigs, on whom six tests can be made, 10 out of 12 can be saved, a considerable advantage when a large number of strains are to be tested.

We have tested by this method 20 non-virulent and 40 virulent strains of diphtheria bacilli, and the results obtained corresponded exactly with those obtained with the subcutaneous test for virulence.

A little practise is required for the proper performance of the injection; experience should also be obtained at first by working with known virulent and non-virulent strains; but with good technique, fresh Loeffler slants, and a proper 24-hour surface growth, the results will be found to correspond exactly with those obtained in the usual and less economical way for determining the virulence of diphtheria and diphtheria-like organisms. This method is being used at present in the routine virulence testing at the Research Laboratory of the New York City Department of Health.

METHODS OF USING DIPHTHERIA TOXIN IN THE SCHICK TEST AND OF CONTROLLING THE REACTION

Abraham Zingher

(*Reprinted from Am. Jour. Dis. Child., April,* 1916)

The practical value that the Schick reaction[1] has acquired in the diagnosis of susceptibility or immunity to diphtheria makes it desirable to say a few words about the toxin used in the test, the heated or overneutralized toxin used in the control test, the technique of the test, and finally about the interpretation of the reactions.

The diphtheria toxin for use in the Schick test consists of a broth culture of the diphtheria bacillus, which has been grown in the thermostat at 37° C. for six days. To kill the living organisms 10 parts of a 5% solution of carbolic acid (0.5%) are then added, and the bacteria allowed to sediment by keeping the broth culture in the ice-box during the following 2-3 days. The supernatent culture fluid is now passed through a Berkefeld filter, and the clear filtrate of toxin standardized. This is only a preliminary standardization. Since a considerable part of the toxin is converted into toxoids during the succeeding 12-18 months, we have to use, for purposes of the Schick test, a toxin that has been ripened for at least a year, and then carefully standardized by determining the M.L.D. of the toxin for a 250 gram guinea-pig. 1/50 of the M.L.D. in 0.2 c.c. of sterile physiological salt solution is injected intracutaneously on the flexor surface of the forearm or arm.

The undiluted diphtheria toxin can be obtained either in bulk (vials of 2 c.c.) or in the single outfits devised by the writer.

The bulk toxin will keep its strength very well, if a ripened toxin is used. A *primary dilution* of the toxin is first made by adding 100 M.L.D. to a sufficient amount of sterile physiological salt solution to make 10 c.c. For instance, if the M.L.D. of the toxin is 0.007 c.c., then 0.7 c.c. of the undiluted toxin is added to 9.3 c.c. of salt solution. Each cubic centimeter of the primary dilution will represent 10 M.L.D. This primary dilution will keep in the ice-box for about 2 weeks. For the Schick test a fresh *final dilution* is made daily, or whenever required, by drawing up with the Record syringe 1.0 c.c. of the primary dilution and adding it to 99 c.c. of sterile salt solution. Several bottles of saline (99 c.c.) may be conveniently kept on hand. The final dilution represents in 1.0 c.c. 1/10 M.L.D., and in 0.2 c.c. 1/50 M.L.D., the amount injected intracutaneously in the test. This method of diluting the toxin is convenient for use in institutions, or in places where the daily carrying out of the test requires that a fresh dilution of toxin be frequently prepared. If the undiluted toxin and the primary dilution are kept very cold (45° F. or less), there will be no danger of deterioration, and the results with the test will be reliable.

In view of the necessity of the general practitioners having a more convenient way of obtaining the undiluted toxin and of diluting it with the proper amount of sterile saline, an outfit has been devised which can be obtained by physicians in New York City from the Bureau of Laboratories of the Department of Health. These outfits are also supplied by several commercial laboratories. The outfit has been described in a previous communication in the J. A. M. A.[2] Owing to a slight modification, the essential parts of the outfit will be given again. In each package there is a capillary tube, which contains a little over 1 M.L.D. of ripened, undiluted diphtheria toxin; a small rubber bulb, similar to the ones used in the vaccine outfits, and a bottle, which contains 10 c.c. of sterile physiological salt solution. To use the outfit one end of the capillary glass tube is broken off, and the end carefully pushed through the neck of the rubber bulb, until it punctures the diaphragm within, and enters the cavity of the bulb; the other end of the tube is then broken off. The bulb is now held between thumb and middle finger, the index finger placed over the opening in the larger end of the bulb and the toxin expelled into the saline. The capillary tube is rinsed out by drawing up saline several times, the bottle is corked and the diluted toxin shaken. Each c.c. of the dilution represents 1/10 M.L.D., and every 0.2 c.c. 1/50 M.L.D., the amount used in the test. The capillary tubes with the toxin may also be obtained in quantities of 6 and 12. By refilling the 10 c.c. bottle with sterile salt solution or with cool boiled water, a fresh dilution can, whenever required, be readily prepared.

The undiluted toxin in the capillaries will deteriorate very little, if care is taken to keep the outfits in a very cold place. The diluted toxin should not be used after 12-24 hours. Each capillary tube contains a small excess of toxin (1¼ M.L.D.), to allow for any possible slight deterioration.

It was with the hope of popularizing a test, which has given us[3] and others[4] such reliable results, especially in children up to the age of 15 years, when pseudo-reactions are rare, that this outfit has been devised and made available for the general practitioner. No stronger proof of the reliability of the reaction need be offered than the fact that, of 800 patients sick with scarlet fever at the Willard Parker Hospital, who had given a negative test on admission, not one developed clinical diphtheria, although no antitoxin had been injected, and about 15% of the children showed, during their stay in the institution, virulent diphtheria bacilli in throat cultures.

The three prerequisites for the test are: (a) a reliable toxin, (b) a proper technique, and (c) a correct interpretation of the reaction. Care in getting and keeping the toxin will answer the first. A good syringe (preferably a "Record") and a fine platinum-iridium needle are needed for the second. The ability to carry out the test properly is easily acquired. One point that may serve in guiding in the injection of the diluted toxin might be emphasized. If the needle has been inserted into the proper layer of the epidermis, then the oval opening of the needle will be visible through the superficial layers of cells. A definite wheal-like elevation, with the distinct markings of the openings of the hair-follicles, shows that the injection has been made properly, and that the fluid is confined to a small area of the epidermis. Here it will exert its irritant action, if the individual tested is not immune to diphtheria.

For the interpretation of the test it is important to remember that the *positive* reaction represents the action of an irritant toxin upon unprotected cells. A trace of redness appears slowly at the site of injection in from 12-24 hours, and usually a distinct reaction in the course of 24-48 hours. The reaction reaches its height on the 3rd or 4th day and gradually disappears, leaving a definitely circumscribed scaling area of brownish pigmentation, which persists for 3-6 weeks. In the *negative* reaction the skin remains normal. In the *pseudo-reaction* there is an anaphylactic response of the tissue cells to the protein substance of the diphtheria bacillus, which is present in the toxic broth used for the test. Like other anaphylactic skin phenomena, the reaction is of an urticarial nature, appears early, within 6-18 hours, reaches its height in 36-48 hours, and disappears on the 3d or 4th day, leaving no pigmentation or only a poorly defined, small brownish spot. At its height the pseudo-reaction shows varying degrees of infiltration, and appears as a small central area of dusky redness, with a secondary areola, which gradually fades into the surrounding skin. A control test may be made by injecting into the other forearm diphtheria toxin which has been heated to 75° C. for 5 minutes or has been overneutralized with antitoxin. This overneutralized toxin is prepared by adding 2 units of antitoxin to each L + dose of toxin. If the first test gave a positive reaction, then the control test will be negative. If it gave a pseudo-reaction, then the control will show a similar area of redness and infiltration, and both reactions will run the same clinical course. Since the free toxin in the solution has been destroyed by heating or neutralized with antitoxin, the only substances that remain are the protein of the autolyzed diphtheria bacilli and the constituents of the nutrient broth. Sterile broth in the same dilution will not as a rule give the pseudo-reactions; in a less dilute condition it will show in some a faint reaction. We must, therefore, conclude that it is the protein of the autolyzed diphtheria bacilli, which is the active agent. Similar and even more intensive pseudo-reactions may be obtained with a dilution of an autolysate of diphtheria bacilli which contains no trace of soluble toxin. Heated diphtheria toxin, for use in the control tests, can be obtained in outfits similar to those for the Schick test.

Occasionally one sees a *combined reaction,* which represents both a positive and a pseudo-reaction. The central area of redness is larger and better defined; the amount of infiltration is also more marked. To interpret such a reaction we should obtain the evidence of a true reaction, a definite area of scaling brownish pigmentation after the pseudo element has disappeared in the Schick test. In addition we should obtain a similar, though weaker, reaction in the control test made with heated or overneutralized toxin. The control will, of course, represent only the pseudo-reaction. These pseudo-reactions are rarely seen in the young, but quite frequently in the adult. Unless one is able to carefully differentiate them from the positive reactions, it will be safer in practice to consider all reactions as positive and treat them as such on exposure to diphtheria.

For purposes of recording the tests, we may designate the positive reaction by the following signs, which represent varying degrees of intensity. This is especially important in the active immunization with mixtures of diphtheria toxin and antitoxin, when we wish to know the degree of the original or control test before the immunizing injections and of tests made at intervals after the injections.

++ Strongly positive. Marked redness and considerable local infiltration, occasionally superficial vesiculation.
+ Positive. Redness and little or no local infiltration.
± Moderately positive. Varying degrees of redness and no local infiltration.
$\underline{\underline{\pm}}$ Faintly positive. Slight redness and no local infiltration.
— Negative. No redness and no local infiltration.

CONCLUSIONS

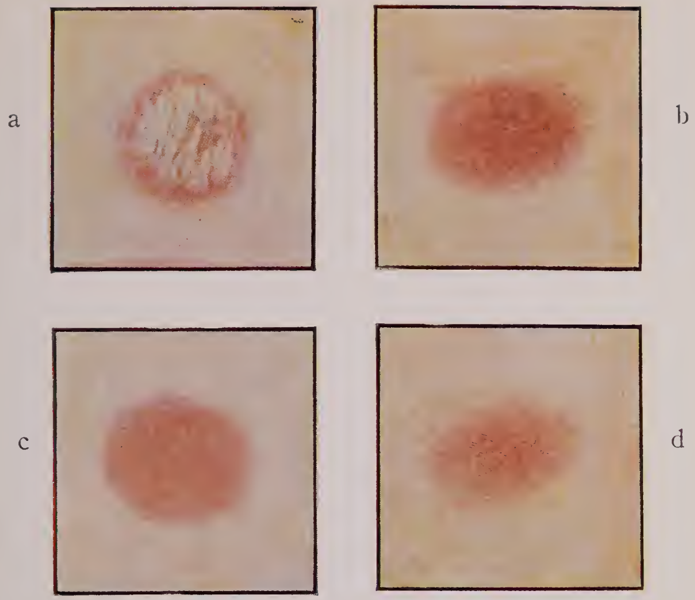
1. The great practical value connected with the Schick test makes it desirable that the results obtained with it should be reliable.

2. The accuracy of the results will depend not only on the toxin, but also on the care with which the test is made and on the interpretation of the reaction.

3. The undiluted toxin is available in bulk or in capillary tubes. It

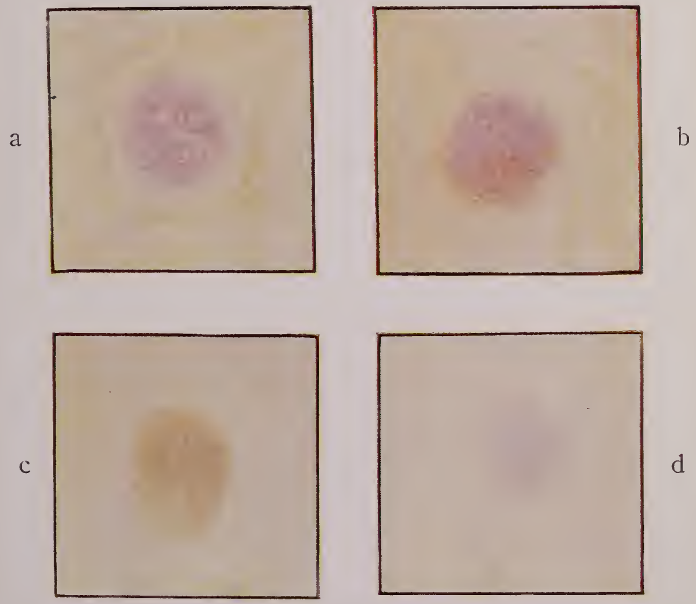
PLATE I

A



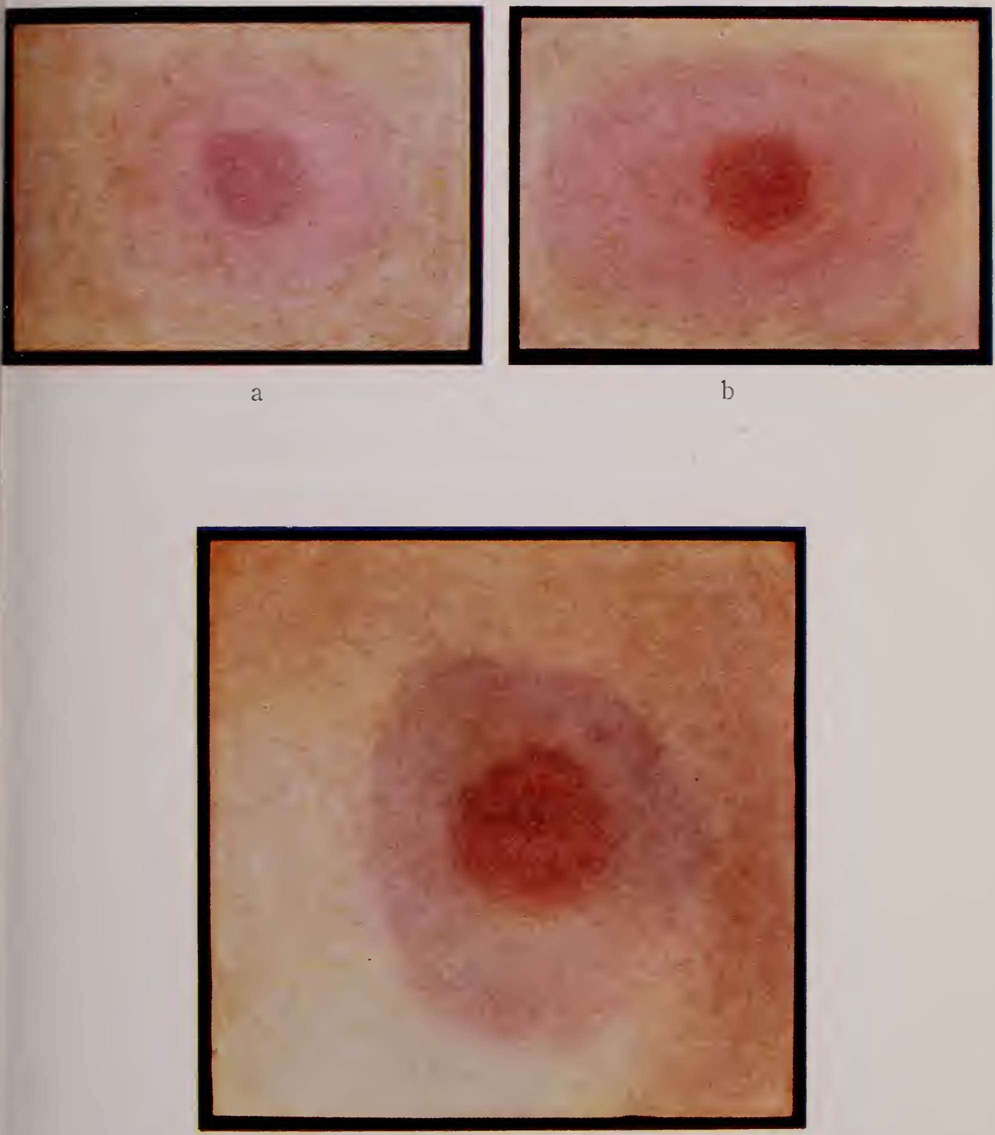
A—Shows four typical positive Schick reactions of varying degrees of intensity forty-eight hours after test; (a) is a strongly positive reaction, with vesiculation of the surface layers of the epithelium, which is seen occasionally in individuals who have practically no antitoxin; (b) and (c) are positive reactions; (d) a moderately positive reaction.

B



B—Shows a fading positive Schick reaction one to four weeks after test in various stages of scaling and pigmentation; (a) shows redness, scaling and beginning pigmentation after one week; (b) and (c) pigmentation after two and three weeks; (d) faint pigmentation after four weeks.

PLATE II



a b

c

Shows two pseudoreactions forty-eight hours after test, and a combined reaction; (a) mild; (b) marked; (c) a combined positive and pseudoreaction.

should be well ripened and always kept very cold and in a dark place. Capillary tubes with heated toxin are available for controlling the Schick test.

4. The positive reaction should be considered as indicating a lack of immunity, unless the pseudo-reaction can be eliminated by a control test. The negative reaction is a definite sign of immunity.

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STUDIES OF LABORATORY MEDIA

Jane L. Berry

The work described has been carried on at intervals over a considerable period. It was begun with the object of studying some of the ordinary types of laboratory media, in order, if possible, to find modifications more favorable for the growth of bacteria, or to find methods which would lessen the cost of production of media, while at the same time leaving their nutritive value for bacteria unchanged.

The chief study has been in connection with the agar used in milk work, for on account of the immense number of milk samples tested the production of the agar used for these tests constitutes one of the important items of our routine laboratory work.

The idea of dilution was one of the first which suggested itself, and a long series of tests were made to ascertain what effect would be produced in milk plating by the use of a dilute agar, that is, agar containing reduced amounts of meat juice, peptone and salt, the amount of the agar itself remaining unchanged. Duplicate, triplicate or quadruplicate plates were made from milk samples in the dilute agar and compared with parallel plates in agar made by the customary standard methods.

It was at once apparent not only that agar containing less of the nutritive elements gave as many colonies with milk samples, but that the colonies were often actually increased in number up to a certain point of agar dilution.

In accordance with the well-known variability of milk samples the above result was not found with every milk tested, but when a large number of plates were compared the increased number of colonies in the weaker agar was very noticeable, and was often found present in a surprising proportion.

With Veal Agar, tried in a series of over one hundred plates, from twenty-nine milk samples, about twice as many colonies were found in the plates made of Veal Agar one-half dilute (containing one-half the amount of meat juice, peptone and salt) as in the corresponding plates made of Veal Agar of the usual strength.

In order to see whether this result was due to any peculiarity of the meat used, supplies of meat for broth were obtained from butchers in three widely separated sections of the city, but it was still found that the agar containing half the amount of broth showed the larger number of milk colonies.

Plain 12 *Agar.*

No further dilution of Veal Agar was tried, but with Meat Extract Agar, called by us Plain Agar, the study was carried much further. Beginning, as before, with a one-half dilution, tests were made with agar containing successive dilutions of one-fourth, one-eighth and one-twelfth the usual amounts of meat extract, peptone and salt. As with Veal Agar, the greater proportion of colonies were found with the more dilute agar, this rule holding good up to the one-eighth dilution. Beyond this point the increased colony production was no longer noticeable.

The greatest number of tests were made with the one-twelfth dilution, known as Plain 12, for on account of the great reduction in cost, it was hoped that it might be found practicable for use in the routine milk examinations. According to tests made in our laboratory by different sets of workers there is practically no difference found between the number of colonies given by milk samples in Plain 12 and in the Standard Meat Extract Agar[1] (Liebig's beef extract).

The objection to the use of the dilute agar consists in a tendency to the formation of smaller colonies. It is found that with a small proportion of milk samples, amounting to about three per cent. in the many hundreds tested by us, this tendency is very marked, making the count more difficult. On the other hand, with many samples, the results in Plain 12 and in Standard Agar are practically identical in every way, and for the general run of milk samples the results are so good in the dilute agar that it would seem to be available if desired as a medium for routine milk work in the event of any change in the standard methods. As the Plain 12 can be made at a cost per litre of four and one-half cents for ingredients as compared with forty-two and one-half cents per litre for the Standard Agar, the saving in expense for the great quantity used would be very marked.

Water Agar.

Carrying the experiment of dilution still further the effect was tried of eliminating meat juice and commercial peptone entirely, a medium which we called Water Agar, being made up with agar, one and one-half per cent., sodium chloride 0.5 per cent., and water. After boiling and clearing in the usual way this gave a clear colorless agar with a reaction either neutral or slightly acid to phenolphthalein.

To our surprise we found that very good plates were obtained in this medium with certain milk samples, the colonies, though small, being sharp and distinct, and often more numerous than on Standard Agar, notwithstanding the small basis for bacterial growth. This result, however, was very irregular, especially as to size and distinctness of colonies, varying greatly with different milk samples and different lots of Water Agar.

Many attempts were made by the addition of different substances to overcome this difficulty, since if that were possible, such agar, on account of ease and cheapness of production, would become an ideal medium for extensive routine work.

Potato juice, dextrose, gelatine and nutrose in varying amounts were successively tried without effect except in the case of the nutrose. This, added in three per cent. and five per cent. amounts, gave considerable improvement, but the results were still too irregular for comparison with those given by Standard Agar.

Pancreatin Agar.

An extended series of tests have been carried out with a medium recommended by Hottinger and called by us Pancreatin Agar. Hottinger (Centralbl. f. Bakt., Dec. 4th, 1912), stating that most of the nutritive elements of meat are lost in the customary process of making laboratory broth, recommends a method of slow digestion by means of the addition of pancreatin, and claims that in this way so much peptone can be obtained from the meat that no addition of commercial peptone is necessary. Sodium chloride is also omitted.

As slightly modified by us the process is as follows:

Meat (veal)	750 gms.
Water	1,500 c. c.

The meat is carefully freed from all fat and fascia, and cut into finger strip pieces. The water is heated to the boiling point, and the meat added, a few pieces at a time, allowing the water to boil up again between each addition, and more strongly for a moment or two when all the meat is added. The kettle is then removed from the fire, the meat is taken out with a fork and passed through the cutting machine. The water is cooled to 37° C. and put into a two litre flask.

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[1] Am. Jour. Public Health, V, 1, Jan., 1915, p. 64; (ibid.), V, 12, Dec., 1915, p. 1261.

Next add:

Sodium carbonate	1½ gm.
Pancreatin (siccum)	3 gms.
Chloroform	10 c. c.
Toluol	10 c. c.

Shake well, add the chopped meat and again shake thoroughly.

Incubate at 37° C. over night.

Shake next day and add more pancreatin if digestion has not actively begun as shown by the yellow color and the decrease in size of the meat particles. After two to three days in the incubator, or four to five days at room temperature with vigorous shaking once or twice a day, the meat will be disintegrated into a finely divided mass with an offensive odor.

If desired, the medium can be stored in the ice-box at this stage, after adding a slight amount of hydrochloric acid if not already acid, and enough chloroform and toluol to prevent putrefaction.

To prepare for use decant the fluid through cheese cloth, add an equal amount of water to the residue and the cheese cloth filter, stir thoroughly, and again filter through cheese cloth; then pour the two filtrates together, pass through cotton and last through filter paper, neutralize to phenolphthalein, put in a flask and autoclave. The product thus obtained is Hottinger's Stock Broth, which he advises diluting "to ten, twenty, thirty litres as desired."

It was tried by us in varying strengths. As before, the colonies were found to increase in number with the weaker broth, but up to a one in fifteen dilution the size of the colonies was only slightly affected. Pancreatin Agar made with this broth dilution, showed colonies in many milk plates fully equalling in size and distinctness those from the same samples on Standard Agar.

These results, verified in many tests, constitute a strong claim in favor of this agar, especially since the cost for materials is only about one-third that of the standard medium. The original Pancreatin Stock Broth is more troublesome to produce, but this is so highly diluted that in the end the difference in the labor required is very slight.

In addition to milk plating, tests were made as to the value of Pancreatin Broth and Agar for general laboratory purposes. They were found to be very serviceable media for carrying on many stock cultures, for typhoid, paratyphoid, coli communis, staphylococcus, streptococcus, pneumococcus, meningococcus, gonococcus, dysentery and glanders carried on in both the broth and the agar for many weeks showed no apparent loss of vigor. In plates the results were not so good, typhoid and coli showing colonies both smaller and less numerous than with agar containing commercial peptone. Diphtheria bacilli could not be cultivated successfully in either Pancreatin Broth of Pancreatin Agar; several strains tried always died out after a few transfers. The whooping cough organism also refused to grow on the pancreatin media.

Used Agar.

Veal Agar which had been used in Blake bottles for the cultivation of different organisms in the making of vaccines was melted, poured together, made neutral to phenolphthalein, sterilized and again planted with various organisms. Except for a loss of transparency, which could probably be restored by fresh clearing and filtering, it was found that the previous use of the agar seemed in no way to affect its value as a culture medium for the ordinary bacteria. Typhoid, paratyphoid, coli communis, staphylococcus, streptococcus, pneumococcus, gonococcus and diphtheria grew as vigorously as on the new agar, and occasionally with a heavier growth. This availability of Used Agar is especially useful where large amounts of media are required, as in the preparation of vaccines for animal inoculation.

Martin's Broth and Martin's Broth Agar.

These media, highly recommended by the French workers and others, in our hands proved good culture media for the growth of the ordinary laboratory organisms, but did not seem to offer sufficient advantages over the ordinary Stock Veal Broth and Agar to compensate for the extra trouble involved in the preparation of the peptone from the pig's stomach.

Ringer's Solution Medium.

This has been recommended by several observers, and especially by Pfeiller and Lentz (Centralbl. p. Bakt. Orig., Feb. 12, 1914). The cost of the Chemicals used in the preparation of Ringer's Solution is so slight that on the score of economy it would far outrank the ordinary laboratory media, but in our hands it has not as a rule given favorable results. While certain tests in plate cultures or on agar slants have shown good growth, yet in general the ordinary stock organisms have grown much less abundantly in Ringer than in Veal or Meat Extract Agar, and on slant agar tubes nearly all the organism tried died out when carried on for successive transfers. Similar results were obtained when Ringer's solution was used as a fluid medium with the addition of peptone.

Peptones.

The rise in price and limitation of the supply of imported peptone, due to the war, led to an investigation of the various brands manufactured in this country.

Tests were carried out by means of three laboratory stock organisms, typhoid, coli communis and streptococcus, plated in agar containing two per cent. of the different peptones, the colonies being compared with those in control plates of agar made with the imported Witte Peptone. The results are shown in the following table:

Agar Plate Tests—Different Domestic Peptones Compared with Witte Peptone

Peptone Tested	Organism	Number Tests	Plates	Total Average Colonies		Appearance of Test Colonies Compared with Control Colonies
				Test Agar	Control Agar	
No. 1	Typhoid	7	18	979	1,044	Equal
	Coli	4	10	640	609	Equal
	Strept.	2	6	755	735	Equal
No. 2	Typhoid	6	18	1,723	1,616	Equal
No. 3	Typhoid	3	9	1,052	1,223	Equal
	Coli	3	9	297	289	Equal
	Strept.	1	3	296	288	Better
No 4	Typhoid	1	3	51	21	Equal
	Coli	2	6	120	127	Equal
	Strept	1	3	366	308	Better
No. 5	Typhoid	1	3	63	81	Not Equal
	Coli	1	3	195	252	Not Equal
	Strept.	1	3	63	60	Not Equal
No. 6	Typhoid	1	3	88	80	Slightly Better
	Coli	1	3	145	270	Slightly Better
	Strept.	1	3	82	63	Better
No. 7	Coli	3	7	529	550	Equal
	Strept.	1	3	550	447	Equal
No. 8	Typhoid	3	9	1,054	1,223	Small and indistinct
	Coli	3	9	274	289	Small and indistinct
	Strept.	1	3	358	288	Small and indistinct
No. 9	Typhoid	4	9	1,310	1,351	Equal
	Coli	3	9	608	665	Equa
No. 10	Typhoid	1	3	25	21	Equal
	Coli	2	6	137	127	Equal
	Strept.	1	3	265	308	Better
No. 11	Typhoid	1	3	67	81	Not Equal
	Coli	1	1	242	252	Not Equal
	Strept.	1	3	53	60	Not Equal

It is evident that with regard to the number of colonies produced there is no practical difference between the majority of the test plates and the control plates.

With regard to size and distinctness of colonies some of the peptones tested gave results decidedly inferior to Witte, but in most cases there was no difference found, and in a few tests there were larger and more distinct colonies with the domestic than with the imported peptone.

A number of tests have also been made of toxin production with Tetanus and Diphtheria grown in broth made with the different peptones. So far the Witte peptone has generally given better toxin than the domestic varieties, but in several cases the difference has been so slight that it could practically be disregarded. Some of the best results have been obtained with a peptone made in this laboratory by Mr. J. P. Atkinson, which in many tests seems to be quite equal to Witte peptone for the production of diphtheria toxin. These tests are not yet completed.

Chemical tests by Dr. E. J. Banzhaf show so far only minor differences in composition between the different peptones, domestic or imported. (See article by Dr. Banzhaf in this volume.)

It seems evident that if the supply of foreign peptone is entirely cut off we shall still be able to obtain several satisfactory substitutes for laboratory use.

Conclusions

It is found that good results are obtained in milk plates with a one-twelfth dilution of Standard Meat Extract Agar; that is, agar in which the meat extract, peptone and salt are used in one-twelfth the usual amount, the proportion of agar remaining unchanged. This medium can be made at a greatly reduced cost and could be used if agreed upon, as a substitute for Standard Agar.

Still better results are given for milk work by agar made with a one to fifteen dilution of Hottinger's Stock Broth. This agar, in which the necessary peptone is obtained by digestion of the meat with pancreatin, can also be made at a much reduced cost, and in addition to its availability for milk work, is a good medium for carrying on many laboratory stock cultures.

Used Agar, agar which has already been used for the cultivation of various organisms, can be melted, poured together, titrated, sterilized and again used as a medium for bacterial growth. This has been found especially useful for the cultivation of large amounts of bacteria in making vaccines for the inoculation of animals.

Tests made of several varieties of peptone manufactured in this country and also of a peptone made by Mr. J. P. Atkinson in this laboratory show results that compare very well with those obtained by the use of the imported Witte peptone.

PRACTICAL OBSERVATIONS ON THE TITRATION AND ADJUSTMENT OF CULTURE MEDIA

Bertha van H. Anthony and Clarence V. Ekroth

When one studies the methods given in the various text-books for the titration and adjustment of culture media, he is struck by the lack of uniformity of opinion. Not only is the beginner in media preparation bewildered, but even the more experienced worker is led into error. The difficulty arises from the fact that the complex nature of the materials dealt with is by no means fully understood even in the most fundamental culture media. In addition to this the changes that occur under even slightly different conditions and treatment are most varying. As a result, each laboratory is compelled to adopt the methods best adapted to its work and requirements, and each laboratory makes changes in these methods as need arises.

The many requests made constantly for information regarding the methods employed in these Laboratories for the titration and adjustment of both general and special culture media, seem to indicate the need for a detailed account of such procedures. In this paper we have tried to incorporate the practical information gained after a number of years experience. In addition, we have described experiments carried on with a view of clearing up, in a systematic manner, certain points upon which little, if any, information is available.

The Standard Method[13-14] of titrating media for water and milk analyses was devised in an attempt to get uniform preparations of media at all times so that comparable results might be obtained.

The directions are as follows:

"Phenolphthalein shall be the standard indicator in obtaining reaction of all media. Turmeric paper possesses similar properties and its use advised where phenolphthalein is not available. Titrations and adjustments of reactions shall be made as follows:

"Put 5 c.c. of media to be titrated in 45 c.c. of distilled water. Boil briskly one minute. Add 1 c.c. phenolphthalein solution (5 grams of commercial salt in one liter of 50 per cent. alcohol). Titrate while hot (preferably while boiling) with twentieth normal caustic soda. A faint but distinct pink marks the true end point. This distinct pink color may be described as a combination of 25 per cent. of red (wave length approximately 658), with 75 per cent. of white as shown by the disks of the color top, described under Records of Tints and Shades of Color, p. 10. (The Standard* color disks used in teaching optics may be used for this purpose.)

"In practice, titration is continued until the pink color of alkaline phenolphthalein matches that of the fused disks. All reactions shall be expressed with reference to the phenolphthalein neutral point and be expressed in percentages of normal acid or alkaline solutions required to neutralize them."

One of the objects of this paper is to consider whether the desired results are actually obtained by the Standard Method or by modifications of this method.

In our laboratory a modification, that is, titration of broth at room temperature (about 20° C.), and of agar at a temperature of about 30° C., has given good results for a number of years past.

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* A small-sized top and disks costing only a few cents may be obtained from Milton Bradley Educational Co., Springfield, Mass.

These and other experiences have led us to investigate further the following subjects:

(a) The effect of prolonged heating on meat infusions and beef extract solutions, as shown by the titration curves of both adjusted and unadjusted portions. The results of boiling samples of media in the casserole for titration.

(b) The adjustment of broth and agar, including the remelting of solid media.

(c) The reaction of peptone solutions and the effects on them of prolonged heating.

(d) The matter of indicators with consideration of the significance and sensitiveness of their end points in different media; also the method of choosing the one most suitable with reference to the hydrogen electrode as a means of selection.

Meat Infusions—It is a well known fact that each time a medium is heated to the boiling point, or above it, the reaction changes and becomes more and more acid, depending on the length of time and the degree of heating. On this subject Eyre[12] says:

"Meat extract [meat infusion] is acid in reaction owing to presence of acid phosphates of potassium and sodium; weak acids of the glycolic series and organic compounds in which an acid character predominates.

"Owing to the nature of the substances from which it derives its reaction, the total acidity of meat extract [infusion] can only be estimated accurately when the solution is at the boiling point. Prolonged boiling [as in media preparation] causes it to undergo hydrolytic changes which increases the acidity."

He states further that meat extract [infusion] becomes stable in reaction after being heated at the boiling point for forty-five minutes so that no additional increase of acidity occurs on further heating.

To procure more definite data as to the effect of heat on the acidity of media the following work was carried out:

Preparation of the Meat Infusions.

Chopped lean veal was soaked over night in tap water‡ in the proportion of one pound of meat to one liter of water. It was then heated at 45°-55° C. for one hour. At this point it was brought to a boil. Then the material (meat and watery infusion) was divided into three lots:

*B30 was kept at the boiling point 30 mins.................. Volumes being made
B60 was kept at the boiling point 60 mins.................. up by addition
B120 was kept at the boiling point 120 mins.................. of tap water.

After being boiled, each lot was strained through cheese cloth, then filtered through paper (S. & S. "Falten" filter) and cotton (first moistened with cold water to hold back the fatty substances).

Each lot was titrated† and then divided again into two parts, one series B 30, B 60, B 120 was run in the autoclave at least six successive times, without the addition of soda.

The second series B 30 C, B 60 C, B 120 C was corrected with normal sodium hydroxid to one per cent. acidity (+1.) and then run in the autoclave with the other set and under the same conditions.

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* Preliminary titrations on samples before boiling had been labelled "A." As these had no significance they are omitted in this article.

† For method of titration followed see page 296.

‡Note.—Weekly analysis of the Croton water supply shows it to contain a negligible amount of mineral matter (only about 40 parts per million, expressed as total hardness). Where the water supply is at all "hard," it is advisable to employ distilled water exclusively.

In all the tests made the autoclaving was done at a pressure between 15 and 17 pounds as indicated by a Bristol Recording Pressure Gauge. The heating was carried on up to a total of eight hours and titrations performed at one-half hour intervals for the first four half hours; then at one hour, two hour and three hour intervals.

After each autoclaving the six samples were titrated and the corrected series B 30 C, B 60 C, etc., were adjusted again when necessary to plus one (+1).

The titrations were performed as follows: Freshly boiled and cooled distilled water was used for all titrations. A five c. c. sample of meat infusion was drawn off by means of a 5 c. c. pipette and added to 45 c. c. of distilled water in a casserole to which 1 c. c. of a 1%* solution of phenolphthalein had been added. While stirring the mixture deci-normal* soda solution from a burette was run in without any heating whatever. The end point taken was the first delicate pink tinge, observable throughout, which did not disappear after stirring the solution and should not disappear for at least one minute. The figures were then recorded. The casserole with the mixture was then set over the flame, brought to a boil and boiled one minute by the watch.

Since the boiling had caused the faint pink color to disappear, the hot mixture was then promptly titrated again, the same end point being approximated† as closely as possible and the figures recorded. The room temperature figure plus that obtained after boiling one minute gave a total which represents the boiling titration figure as recorded in the charts.

In the first lot of veal (series B 30, B 60, B 120), the corrections were made to plus one at the room temperature figures. A second lot of veal infusions ($B_2$30, $B_2$60, $B_2$120) were prepared in the same manner as above. The corrections in this lot were made to plus one at the boiling figures.

DESCRIPTION OF CHARTS

In Charts 1 and 2 are shown the uncorrected portions of meat infusions titrated after successive heatings in the autoclave and plotted according to both the room temperature and the boiling temperature figures. It will be seen that in each lot of meat the room temperature titrations fall into one group, and the boiling titrations in another; also that the boiling figures are the higher.

Chart 3 is a sample chart‡ showing not only the uncorrected portion of B 120 as given in Chart 1, but also the corrected portion, B 120 C. This portion was corrected to plus one (+ 1) according to the room temperature titration figures and readjusted to plus one after each autoclaving, as shown by the α line. The α' line shows the figures of this same material when boiled one minute in the casserole and titrated hot. This line is hypothetical and shows only the amount of soda that would have been needed had the boiling figures been used for adjustment to plus one.

The Θ line shows the total amount of acidity produced in the corrected portion even after the addition of soda according to the room temperature figures.

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* These are also variations from the standard method of using half per cent. solution of phenolphthalein and twentieth normal sodium hydroxid solution. They are, however, in accordance with the methods in use for years in this laboratory, and to preserve uniformity they were adhered to.

† The difficulty of catching the first color change of phenolphthalein in hot solutions will be discussed under Indicators, page 302.

‡ This chart is typical also of the B 30 and B 60 sets.

In Chart 4[*] is represented $B_2$120 (second lot of veal), just as B 120 is shown in Chart 3, except that here the corrected portion ($B_2$120 C β') was adjusted to plus one (+ 1), according to the boiling titration figures. The β line shows the figures obtained each time at room temperature before the sample was boiled one minute in the casserole to give the boiling titration figures. The room temperature curve is inserted here for comparison.

The Θ' line shows the total acidity produced in the portion corrected according to the boiling titration figures.

In Chart 5 all the total acidity lines of the various corrected portions are compared. As with the uncorrected portions, noted in Charts 1 and 2, the total acidity lines fall into two groups according to the method of titration.

If Chart 5 showing the total rises in acidity of the series B 30 C, B 60 C, B 120 C and the series $B_2$30 C, $B_2$60 C, $B_2$120 C were applied successively to the corresponding curves of the uncorrected series in Charts 1 and 2, so that the point of origin in each set were the same, it would be seen that in every instance the total rise of acidity of the corrected curve equals or exceeds that of the uncorrected portion.

From this it is plainly evident that, in spite of successive corrections of acidity with normal sodium hydroxid, hydrolysis not only continues on the application of heat but there is produced in meat infusion media approximately as much acidity as would be developed were no correction made.

Chart 6 shows the actual acidity of the different corrected portions after the successive adjustments and periods of heating. As can be seen, those portions adjusted according to the room temperature titration are in general nearer to the desired reaction of plus one (+1.), especially in the first two half hours—the periods of time of greater practical interest.

In Chart 7 are shown not only the uncorrected portions of B_1 60 and γ') and B_1 120 (γ and γ'), (duplicate material from first lot of veal), but the effect on the corrected portions of the addition of too much normal sodium hydroxid. By mistake after the first half hour in the autoclave and the subsequent titration, there was added to B_1 60 C β' approximately five times the amount of normal sodium hydroxid necessary to correct it to plus one, and to B_1 120 C β' also about five times the right amount. These amounts were in accordance with the boiling titration figures in both cases. The quantities needed for correction were, in round numbers, twice as much for the latter as for the former. The relation of the quantities of alkali added may, therefore, be expressed by the numerical relation of ten to five. The portions of heat infusion (without further additions of soda) were then run as usual in the autoclave with the uncorrected portions and titrated at the same intervals.

Curiosity led us to continue these tests rather than discard them. In consequence, an interesting fact was brought out. To our surprise both over corrected portions recovered the acidity plus one (and more) but at different intervals of time. B_1 60 C β' gained plus one at the end of the fifth hour while B_1 120 β' reached plus one at about the seventh hour.

In all the tests the curves show a distinct and steady rise in acidity. This rise is due to hydrolysis caused by heat and increases continually as more heat[†] is applied. Further, it is plainly evident that a preliminary heating to the boiling point for at least forty-five minutes as advocated by Eyre[12] does not produce a stable reaction uninfluenced by further heating.

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[*] This chart is typical also of the B_2 30 and B_2 60 sets.

[†] Three of the uncorrected portions, B_2 30, B_2 60, B_2 120, were run an additional six hours, making fourteen hours all told in the autoclave at fifteen pounds pressure. The results are shown in last columns of Table I.

TABLE I

Meat Infusion	Reaction Before Autoclaving		After 8 Hours in Autoclave		After 15 Hours in Autoclave	
	R. T.*	B. T.†	R. T.	B. T.	R. T.	B. T.
$B_2$30	1.7	2.7	4.9	6.5	7.4	10.5
$B_2$60	1.9	2.9	4.6	6.3	7.3	10.4
$B_2$120	2.1	3.1	4.3	6.5	6.9	9.3

* R. T. = room temperature titration.
†B. T. = boiling temperature titration.

In the above tests we have gone outside the limits of interest from the practical standpoint. We were led to this, however, in an attempt to locate the point of complete hydrolysis or maximum acidity of meat infusions. This goal was not reached, as stated above, even after fourteen hours autoclaving. We are continuing this work.

In the usual preparation of media from meat the total amount of heating in the autoclave varies from one-half hour, at about fifteen pounds pressure, for sterilization of ordinary broth, to two and one-half hours in the preparation and sterilization of agar.

It developed in the tests made on the corrected meat infusions that the change in acidity in the first half hour in the autoclave (the usual time for the sterilization of finished media) varied from nothing to 0.3 at the room temperature figures, while at boiling temperature figures the change in acidity was 0.3 to 0.4%. (See Chart 6.)

The nature of the acid products which are formed as a result of the hydrolytic decomposition on boiling with sodium hydroxid may be different from those produced by hydrolysis alone when boiling unadjusted media. Therefore while the reaction may be adjusted in each to the same point of acidity, the behavior of the media toward the various organisms may not be the same.

Similar tests to those above were made on Liebig's beef extract dissolved in tap water, filtered and titrated in a similar fashion. These tests showed no change in the reaction of the uncorrected series even after eight hours heating. Although a beef extract solution is undoubtedly quite stable*, as compared with meat infusions, a sample corrected† to neutral, after two hours in the autoclave, rose two-tenths per cent. in acidity. This was corrected to neutral once more and did not change again, although heated four hours longer.

While the change above is almost negligible the addition of peptone to this, as to other media, raises the change in acidity further. This must be taken into consideration in the preparation of media with beef extract when delicate end points are desired.

Broth‡—The tests on the meat infusions were carried out before any peptone or salt had been added. In the preparation of broth the choice of titration methods must of course be governed by the manner of preparing the meat juice in the preliminary steps. The relative merits of pressing out the meat juice before or after heating the soaked meat must be determined by experiment in the kinds of work for which the media are destined.

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* This stability is due probably to very prolonged heating in the preparation of the beef extract itself.

† Correction based on room temperature titration.

‡Broth=meat infusion plus peptone and usually salt.

For those workers whose needs and experience lead them to express the meat juice in the cold state and then dissolve the peptone and salt with very little preliminary heating, the use of the boiling titration for correction is essential, in order to approximate the future conditions due to further heating and sterilization after the reaction of the batch of medium has been set.

With us the best toxin production has seemed to be obtained when the meat juice is pressed out after heating large amounts (20 liters) for one hour at 45°–55° C. and then boiling up strongly until the meat coagulates. The meat infusion* is then strained through cheese cloth. After the requisite amount of peptone and salt have been dissolved by further heating up to the boiling point and the mixture boiled one half hour, the reaction is set according to a room temperature titration.

If the specimen is boiled† in the casserole before titration, it no longer represents the lot of broth in the kettle but has risen somewhat in acidity. Consequently, if the large lot be adjusted according to the boiling titration a false correction is made. To be sure, after adding the normal soda solution, any further boiling of the large lot, together with the final sterilization, raises the acidity but not to just the desired point as shown in the tests on meat infusions (Chart 6).

For example, the final reaction of +1.2 in the case of diphtheria toxin broth, is found to be more uniformly obtained by titrating at room temperature and setting the reaction to +1. This allows 0.2 rise due to heating if the broth is to be sterilized at 15 pounds pressure (121.6° C.) for one-half hour. If the sterilization is to be carried on at only five pounds pressure (108.8° C.) for one hour on three successive days, or in the Arnold sterilizer, streaming steam (100° C.) for the same length of time, the reaction is set at +1.1, as the more moderate heating raises the broth only about 0.1 per cent. in acidity, making the finished product +1.2 in reaction. The use of this method for the diphtheria and tetanus toxin broths for a number of years has shown fully its value, and it is the method still employed by us.

On the other hand, Eyre states that the correct estimation of acidity present can be made only by titration at the boiling point.

Judged from our results as shown in Chart 4 by the β^1 and β curves, this statement is erroneous. The β^1 curve shows the reactions and successive adjustments to plus one (+1) based upon the *boiling temperature* figures. The reactions at room temperature of this material are shown by the β curve. This latter is far below the β^1 curve (from 0.5 per cent. to 1 per cent.), and gives the actual reaction of the medium at a temperature nearer that of the incubator (37.5° C.).‡

To make this point clear, let us assume for example that a medium is to be adjusted to a definite acidity of +1. according to the boiling titration, as stated in the Standard Method. The real reaction at which the bacteria will then be grown in the incubator is not that indicated by the boiling titration figure but a reaction which is lower in acidity to an extent of about one per cent.—in other words, almost neutral.

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* This method proves useful in other lines of work, for at this point the infusion can be filtered, sterilized and stored. It is ready for further use on the addition of any suitable peptone and may be set at any desired reaction; or it is ready as a basis for making agar.

† At this point may be mentioned the length of time recommended for boiling by different authors. Heineman (6) heats "to boiling." Jordan (4), MacNeal (9) and the Standard Method (14) boil one minute. Park and Williams (5) (1914) boil two minutes. Abbott (8), Abel (7), Hiss and Zinsser (2), Mallory and Wright (11), Swithinbank and Newman (10) boil for three minutes.

‡ It is only after four hours autoclaving that the room temperature reaction of plus one (+1) is reached.

On the other hand, in Chart 3 (α^1 curve), there is shown a similar meat infusion, adjusted to plus one (+1) at *room temperature titration.* The boiling titration figures are plotted as curve α^1. The cold titration curve (α) comes so near the desired reaction of plus one (+1) that even a mere glance will suffice to convince one that the room temperature titration approaches more closely the one per cent. line, which is the acidity we set out to secure.

This was not surprising to us as our practical experience for several years past had indicated such a condition. The modification (page 296) devised at that time and now further substantiated by these experiments, has proved to be so very useful in its results that it is employed in these laboratories for nearly all the routine preparation of some fifty different kinds of media, aggregating over 8,000 liters per year.

Sterile Soda.—In order to avoid the complications of further hydrolysis and precipitation after the addition of soda to a medium which must then be sterilized, it has been suggested that sterilization be done first and that sterile soda be carefully added afterward according to the titration of samples withdrawn under sterile conditions. This has been practised by some workers with success, apparently. So far, in our laboratory, it has shown no advantages in the production of diphtheria toxin broth. Further work in this line is contemplated.

Agar.—When the need arose of supplying large amounts of neutral veal agar for the growing of the gonococcus, streptococcus and other organisms in bulk for antigens, difficulty was experienced. To grow these organisms in large lots with unfailing success is not always easy. Our trouble seemed due chiefly to the reaction of the medium. Finally the modified titration method was adopted for agar also.

Since agar solidifies at a little below 40° C. the room temperature titration was not suitable. At first any temperature between 40° and 50° C. was used. This was reduced later to about 30° C.* Five cubic centimeters of the hot agar are added by means of a pipet to 45 c.c. of distilled water—temperature about 30° C. (verified with a thermometer). One c.c. of phenolphthalein (one per cent. solution in 50 per cent. alcohol) is added and the titration performed at once.

It is now our custom to titrate each batch of neutral veal agar at least twice during its preparation, making the necessary adjustments of reaction. Here, as with the broth, allowance must be made for further heating in the autoclave. Experience shows that agar made from meat infusion rises in acidity usually about three to four-tenths per cent. at 15 pounds pressure during one and one-half to two hours.† Therefore, three to four c.c. per liter of normal soda should be added in excess of the amount required to secure the phenolphthalein neutral point at the time of the first titration. The second titration is made just after filtration, before tubing and sterilizing. If the amount of soda needed does not exceed two-tenths of a per cent., little if any precipitate occurs on heating further to sterilize. If more than the above amount is needed in adjusting the reaction, the medium should be heated in the Arnold for half an hour and the precipitate filtered out, before tubing and sterilizing.

In very particular work the medium is also titrated a third time as it comes from the autoclave. For the last test a tube or small bottle of neutralized glassware should be used in order that the reaction of the agar be unaffected by its container. This sample is tested before it hardens—as remelting would raise its acidity further. On the addition of phenolphthalein, it should show a very delicate shade of pink if it is " neutral."

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* This slight difference of temperature had no noticeable effect on the results of the titration.

† This time is necessary for melting (and clearing with egg) of large batches of agar (5 to 12 liters).

Remelting of Solid Media.—An important factor to be considered in the adjustment of media is the remelting of solid media for the addition of sterile substances such as blood, serum, etc., or for the purpose of immediate use in plating.

If, for example, the whole is to be neutral to phenolphthalein when entirely finished an over neutralization is necessary to allow for the acid changes during the re-heating, as in making Bordet Gengou medium.

Since, in spite of the addition of soda for the correction of a medium further hydrolysis occurs when heat is applied, especially in the autoclave, it is impossible to know the exact reaction a medium will have when sterilization is complete or when the medium is remelted. In practical work, however, it has been found that an over neutralization of one-tenth to three-tenths per cent. has given good results when the titration is performed at 30° C.

The *re-sterilization* of media without suitable correction to allow for the effects of heating is to be avoided if a very definite end point is desired.

Peptones.—The present necessity of finding substitutes for Witte's peptone, so long the standard in bacteriological work, has led us to test the reaction of various peptones on the market. A one per cent. solution of each in distilled water was boiled one minute and then filtered through cotton and filter paper. When cool, each was titrated at room temperature and then again, after the same sample had been boiled one minute, that is, the same procedure was followed as in titrating the meat infusions (page 296).

The following table shows the reactions of eight peptones, including Witte's:

REACTION OF PEPTONES

(Titrated with Phenolphthalein)

Kind	Room Temp. Figure (at 20° C.)	Rise After Being Boiled 1 Minute in Casserole	Boiling Temp. Figure
Armour	+0.6	+0.4	+1.0
Atkinson	+0.4	+0.2	+0.6
Difco	+0.6	+0.2	+0.8
Eimer & Amend	+1.0	+0.4	+1.4
Fairchild Culture	+0.7	+0.4	+1.1
Leitz	+0.4	+0.3	+0.7
Squibb	+0.3	+0.1	+0.4
Witte	+0.3	+0.1	+0.4

The peptone solutions were then divided into two sets (as with the meat infusions), one corrected and the other uncorrected. These were given successive runnings in the autoclave and titrated at the same intervals. As with the meat infusions there was a steady increase in acidity though not in so marked a degree. Three uncorrected portions of peptone solutions (Fairchild's, Eimer & Amend's, Squibb's) were also run an additional six hours, making fourteen hours in all in the autoclave. As with the meat infusions the limit of hydrolysis was not reached.

In the corrected portions the total amount of acidity, developed by heating after successive additions of normal soda, again paralleled closely the rise in acidity of the corresponding uncorrected portions.

From the above it is apparent that the introduction of a peptone into a medium will affect the reaction of the whole to some extent.

Indicators.—The shade of phenolphthalein suitable for a correct end point varies greatly in the opinion of different authors.

Miur & Ritchie[1]—give " the first trace of pink."
Hiss & Zinsser[2]—" faint but clear and distinct pink."
Stitt[3]—" a delicate pink (hot titration), a purplish violet color (cold titration)."
Jordan[4]—" Faint but distinct pink color."
Park & Williams, 5th ed., 1914[5]—" Faint, but distinct pink which remains on re-heating."
Heinemann[6]—" Faint but decided and stable pink."
Abel[7]—" brilliant red." (Translation.)
Abbott[8]—" pink color."
MacNeal[9]—" faint but distinct and permanent pink."
Swithinbank & Newman[10]—" clear bright pink color."
Mallory & Wright[11]—" bright pink color," not " the pinkish darkening of the fluid which precedes it."
Eyre[12] uses a " pinkish tinge " or " a faint rose-pink which cooled to 30 degrees or 20 degrees C. becomes more distinct and decidedly deeper and brighter " resembling a " deep magenta color."

These shades given differ more or less from the Standard Method[12].

That no two people seem to titrate to exactly the same shade has often been shown in our laboratory when a different worker in the media room has attempted to set the final reaction of some very particular medium. If the method of titrating is to be at all accurate, it is necessary to *assume* a shade of pink for phenolphthalein. This necessity is brought out by such great discrepancies between different workers titrating the same substance as are given by Clark.* As a help in this, a practical color scale may be of aid in determining the most suitable shade for a certain kind of work and approximated as closely as possible. This should eliminate the personal factor to some extent.

As stated before, in our opinion, the correct shade for a delicate end point in pale broth or other solutions with little color is the first most delicate pink tinge observable throughout, remaining at least one minute.

With us, when titrating agar, a mixture of 5/20 red, 3/20 orange and 12/20 white on the color-top (see page 294) has proved to be a desirable shade for the first and second titrations of neutral agar†; while the third titration, when the medium comes out of the autoclave, should give (on the addition of phenolphthalein) a shade consisting of 3/20 red, 3/20 orange and 14/20 white on the color-top. These shades differ from the ones given above both in the Standard Method of titrating at boiling point (page 294) and our own definition (page 296), but they have yielded very good results. However, it may be as difficult to decide on an end point by means of a color-top or scale as to imagine an end point from the descriptions of the various writers.

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* (16) page 117—Such relative great discrepancies are surprising especially among the chemists.

† The deeper color of agar as compared with the usual color of broth makes necessary the use of some orange in this scale.

Litmus.—Since any medium with meat infusion as a basis and peptone added is a most complex mixture, no one indicator shows all of its varying acid constituents. In the last 10 or 15 years phenolphthalein has been employed largely for it seems to be the most useful, yet it cannot be relied upon in every instance.

Park & Williams* state:

> " Different indicators differ not only in delicacy but in the substances to which they react. A medium alkaline to litmus is acid to phenolphthalein showing that there are present substances possessing a character which litmus does not detect,—weak organic acids and organic compounds—theoretically amphoteric but in which an acid character predominates. Thus a liter of bouillon becomes, on the addition of 1 per cent. of peptone, more alkaline to litmus but decidedly more acid to phenolphthalein; 1000 c.c. of water with 1 per cent. peptone is acid to phenolphthalein to such an extent that 3.5 c. c. of deci-normal NaOH is required to neutralize it. To litmus it is alkaline and requires 3.4 c. c. of deci-normal HCl. Two per cent. peptone doubles the difference. The same figures hold approximately true for peptone broth."

Eyre[12] states that although meat infusion is always acid to phenolphthalein it may react neutral or even alkaline to litmus; again, if rendered exactly neutral to litmus, it still reacts acid to phenolphthalein; that this is due to the facts:

> " (1) Litmus is insensitive to weak organic acids whose presence is readily indicated by phenolphthalein.
>
> " (2) Dibasic sodium phosphate, which is formed during process of neutralization, is a salt which reacts alkaline to litmus but neutral to phenolphthalein."

On the other hand, MacNeal[9] considers litmus the more useful:

> " The neutral point indicated by litmus is very nearly the actual point in respect to acidity and akalinity, and this point is not appreciably displaced in either direction by the addition of a neutral mixture of a feebly dissociated acid and its salts to the solution. The end reaction indicated by phenolphthalein when it turns pink is actually a point at which there is a slight excess of alkali. This is so nearly the neutral point in inorganic solutions, when electrolytic dissociation is marked, that the error is not appreciable. In solutions of organic substances, especially when considerable amounts of feebly dissociated substances such as are contained in peptone or gelatin, are present, this error becomes very appreciable. The discrepancy between the end point for litmus and for phenolphthalein will vary for different lots of media."

Naturally those media which contain litmus as an indicator to show acid production by the growth of bacteria, must be alkaline to litmus yet not too alkaline or the indicator is rendered useless. The testing of such media by the use of litmus paper is an unsatisfactory and crude method useful for only the roughest work. The use of a litmus solution[9] (Merck's purified in five per cent. aqueous solution) is far more satisfactory.

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* 3d and 4th editions, 1908 and 1910.

"Neutral to litmus" is "so and so" acid to phenolphthalein, the figure given varying with the writer. Muir and Ritchie place it at +2.5, Stitt at +1.5 boiling titration and about +0.7 with the cold titration. Abbott gives +2.5, Abel +1.5 to +2.5 and Heinemann +2, all depending on the shade of pink considered by the worker as suitable and the length of time the sample is boiled in the casserole.

In the modified method used in our laboratory, the figure is as low as +0.6 or +0.7 with media prepared with 1 per cent. peptone. (This figure rises to +1. when the boiling titration is used.) A 1 per cent. peptone (Witte) solution in water is about +0.2 to +0.3 with phenolphthalein at room temperature and about +0.4 at boiling figure.

It has been shown by Hildebrand[15] and others[16], [17], that an indicator will not necessarily indicate the point of actual neutrality, but merely a definite degree of hydrogen ion concentration. Where these two points coincide, *i. e.,* where the hydrogen ion concentration at which the indicator changes color, is within the zone of absolute neutrality for a particular mixture of substances, this change of color in an indicator will, of course, be of significance. It would therefore seem desirable to select a specific indicator for each class of media. This could be accomplished only by determining the effect of added alkali upon the electrical conductivity of the particular medium. Such measurement should be made by means of the hydrogen electrode. The measurements of potential are usually expressed in terms of hydrogen ion concentration, that is, acidity.

For an apparatus as described by Bovie[17] the details of manipulation are as follows: A mixture of the medium with water in the same proportions as used for ordinary titrations is placed in a beaker kept at 30° C., and the precaution of excluding CO_2 observed. The indicator is added and the standard electrodes are immersed. Successive portions of decinormal sodium hydroxid solution are then added. After each addition of the alkali the potential of the mixture is observed and recorded.

The point at which the indicator gives its first change of color is marked. The additions of alkali should be continued and the potentials further noted until the curve changes its shape—that is, from concave to convex or vice versa. The actual point at which this change takes place is known as the point of inflection and is a true neutral point. The nearness of the indicator's point of change to this point of inflection determines its suitability for this particular class of media. The indicator in which the change comes nearest to this point should be selected for practical use. Work in choosing such indicators according to this method is planned.

When titrations are performed under ordinary circumstances at the boiling point, it is noticeable that making a decision as to the correct end point of phenolphthalein is much more difficult than when the temperature of 20°-30° C. is used. This is due to the changes of ionization in the mixture caused by this considerable change in temperature.

Besides this the constant presence of colloidal substances* in peptones,† phosphates and sugars in all media gives rise to a further depression of ionization, especially when these substances are decomposed by boiling.

The meagre results from the few investigations conducted in this field lead us to suspect that the presence of sugars in media may have a considerable influence on the effective acidity, that is, the dissociated acid principles[15]. Work in these lines is to be continued.

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* Hildebrand (15), Clark (16) and others have shown that colloidal substances affect the sharpness of indicator end points.

† In the titration of the peptone solutions it was very difficult to get a sharp end point even at 20° C. The indicator (phenolphthalein) seemed to "flare" suddenly from the colorless state to a deeper pink than the one showing a really delicate end point.

SUMMARY

Marked and continued hydrolysis resulting in the formation of acid principles, occurred on successive heatings of meat infusion in the autoclave. The state of complete hydrolysis, *i. e.,* the point at which no further acidity is produced, was not reached with meat infusions (which had been subjected previously to boiling over the open flame for one to two hours) even after prolonged autoclaving at fifteen pounds pressure for eight* hours.

Hydrolysis did not occur in solutions of Liebig's beef extract subjected to similar heatings. In the portion which had been adjusted to neutral, however, slight hydrolysis did take place.

Those portions of meat infusion in which the natural acidity had been adjusted with normal sodium hydroxid to plus one (phenolphthalein) showed that hydrolysis occurred on application of heat and continued to do so after successive adjustments and autoclavings. There was produced in these portions as much acidity, approximately, as was developed on heating the corresponding uncorrected portions.

The further production of acidity after the addition of sodium hydroxid is due to the hydrolytic effect of heat in the presence of water, upon those portions of the medium unbound by alkali.

The fact that hydrolysis is promoted by heat makes inaccurate the estimation of acid ions in a batch of medium when there is taken as an index a sample† titrated at the boiling point with phenolphthalein as the indicator. Boiling has also a marked effect on the ionic concentration in media mixtures, hence the boiling titration called for in the Standard Method and followed more or less closely by many workers and recommended in the various text books, is subject to greater error than titrations performed at a temperature of 20°-30° C.

In the adjustment of culture media not only is the desired end point more closely approximated by titration at 20°-30° C. (see Chart 6), but the misleading information of the boiling titration is avoided (see Chart 4). Although according to Eyre (see page 295) certain acids are detected only at the boiling point, the *actual* reaction of media at the temperatures at which they are *used* is the object of vital importance. The conditions prevailing at these temperatures (incubator, 37.5° C., for some organisms and room temperature for others) are more closely indicated by titrations conducted at room temperature.

As shown in Chart 4 and also in the text of page 299, the actual reaction of a medium titrated by the boiling method is really from ½ per cent. to 1 per cent. lower than is indicated by the boiling titration figures.

The tests made on the reactions towards phenolphthalein of the various peptones on the market showed them to differ greatly in acidity. The effect on peptones of prolonged heating, in tests similar to those on the meat infusions, showed that hydrolysis occurred on the application of heat. Also the development of acidity took place after successive adjustments of reaction with sodium hydroxid, as with the meat infusions and the total amount of hydrolysis approximated closely that of the uncorrected portions.

Here, too, prolonged heating of fourteen hours in the autoclave did not give complete hydrolysis.

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* With three of the meat infusions the time was extended from eight to fourteen hours.

† That is, if the titration is performed after a preliminary boiling of the whole batch of meat infusion. When a titration is made on meat juice pressed out in the cold and containing added peptone dissolved at a low temperature, the boiling of the sample in the casserole is necessary to approximate future conditions after the boiling of the whole batch.

In the usual titration methods no one indicator gives all the evidence desired in every case, *e. g.,* the differences between litmus and phenolphthalein.

The necessary dependence on the change in color of an indicator to show the reaction of a solution, especially in the case of culture media, gives rise to some error. This is due partly to the depressing effect of colloids, phosphates and some sugars which affect the sensitiveness of indicators; and also to variations of judgment with different workers as to the correct shade of color for an end point. Where it is desirable to avoid these influences titrations may be made by measurement of the electrical potential.

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NOMENCLATURE

MEAT INFUSIONS 1st lot of veal	B 30	=preliminary boiling of 30 minutes	
	B 60	=preliminary boiling of 60 minutes	
	B 120	=preliminary boiling of 120 minutes	
	B 30 C	=preliminary boiling of 30 minutes	Corrected to plus one according to room temperature titration after each autoclaving.
	B 60 C	=preliminary boiling of 60 minutes	
	B 120 C	=preliminary boiling of 120 minutes	
	B_1 60	=preliminary boiling of 60 minutes	
	$B_1$120	=preliminary boiling of 120 minutes	
	B_1 60 C	=preliminary boiling of 60 minutes	Corrected to plus one according to boiling temperature titration after the *first* autoclaving. No further adjustments were made because of error in adding too much soda.
	$B_1$120 C	=preliminary boiling of 120 minutes	
MEAT INFUSIONS 2nd lot of veal	B_2 30	=preliminary boiling of 30 minutes	
	B_2 60	=preliminary boiling of 60 minutes	
	$B_2$120	=preliminary boiling of 120 minutes	
	B_2 30 C	=preliminary boiling of 30 minutes	Corrected to plus one according to boiling temperature titration after each autoclaving.
	B_2 60 C	=preliminary boiling of 60 minutes	
	$B_2$120 C	=preliminary boiling of 120 minutes	

KEY TO CURVE NOTATION.

LETTER	LINE	DENOTES
α	—— —— —— ——	= corrected at room temperature
α'	—— —— · —— —— · —— ——	= " " " "(boiling figures)
β'	——— ——— · ——— ———	= " " boiling temperature
β	—— ——·· —— ——·· —— ——	= " " "(room temp. figures)
γ	————————————	= uncorrected (room temperature)
γ'	- - - - - - - - - - -	= " (boiling temperature)
θ	——— · ——— · ——— · ——	= Total acidity, corrected at room temp.
θ'	——— ·· ——— ·· ——— ···	= " " " " boiling temp.

CHART 1.

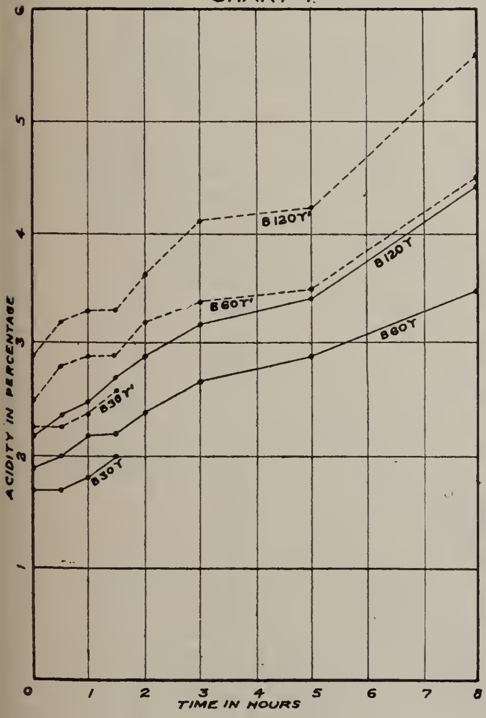


CHART 2.

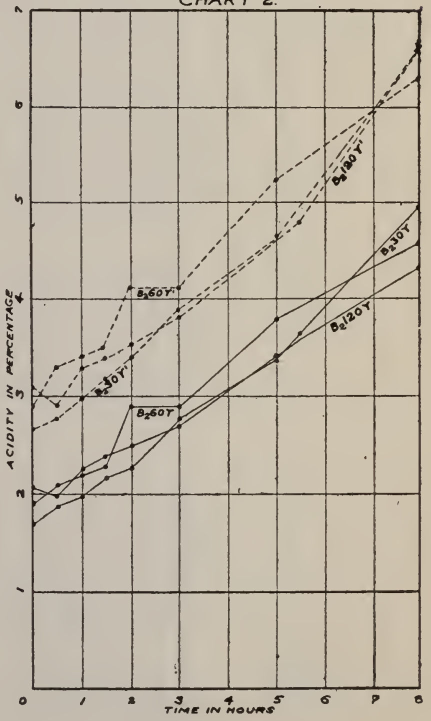


CHART 3.

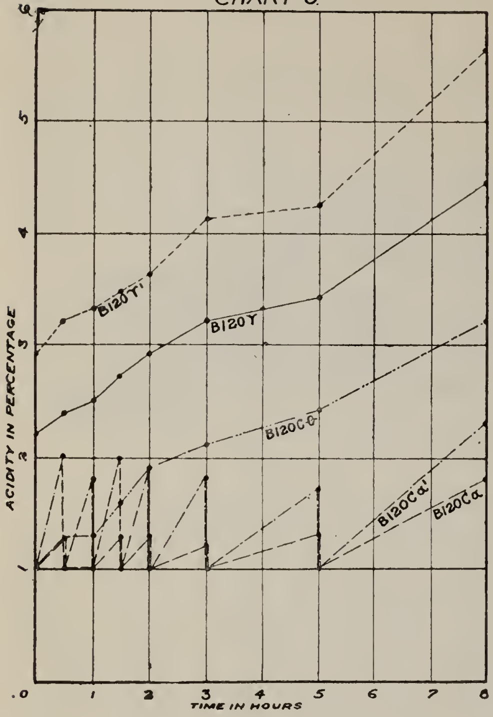


CHART 4

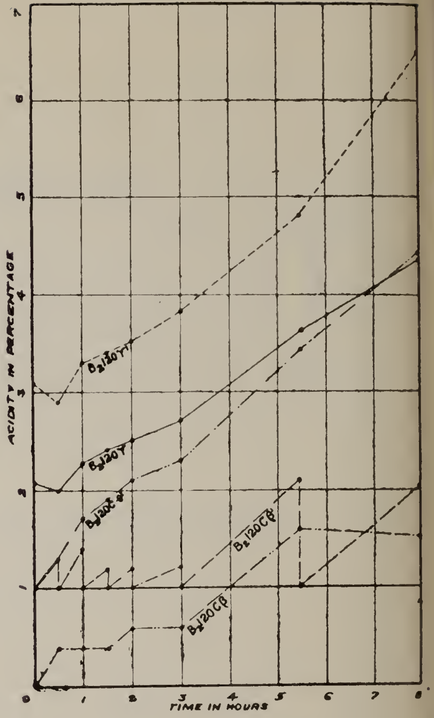
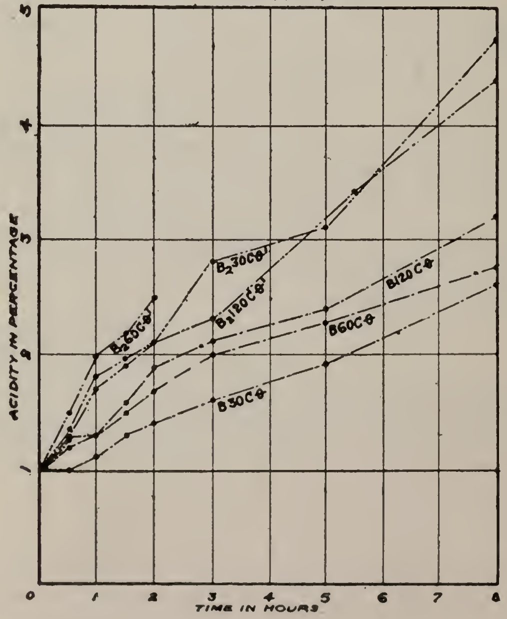


CHART 5.



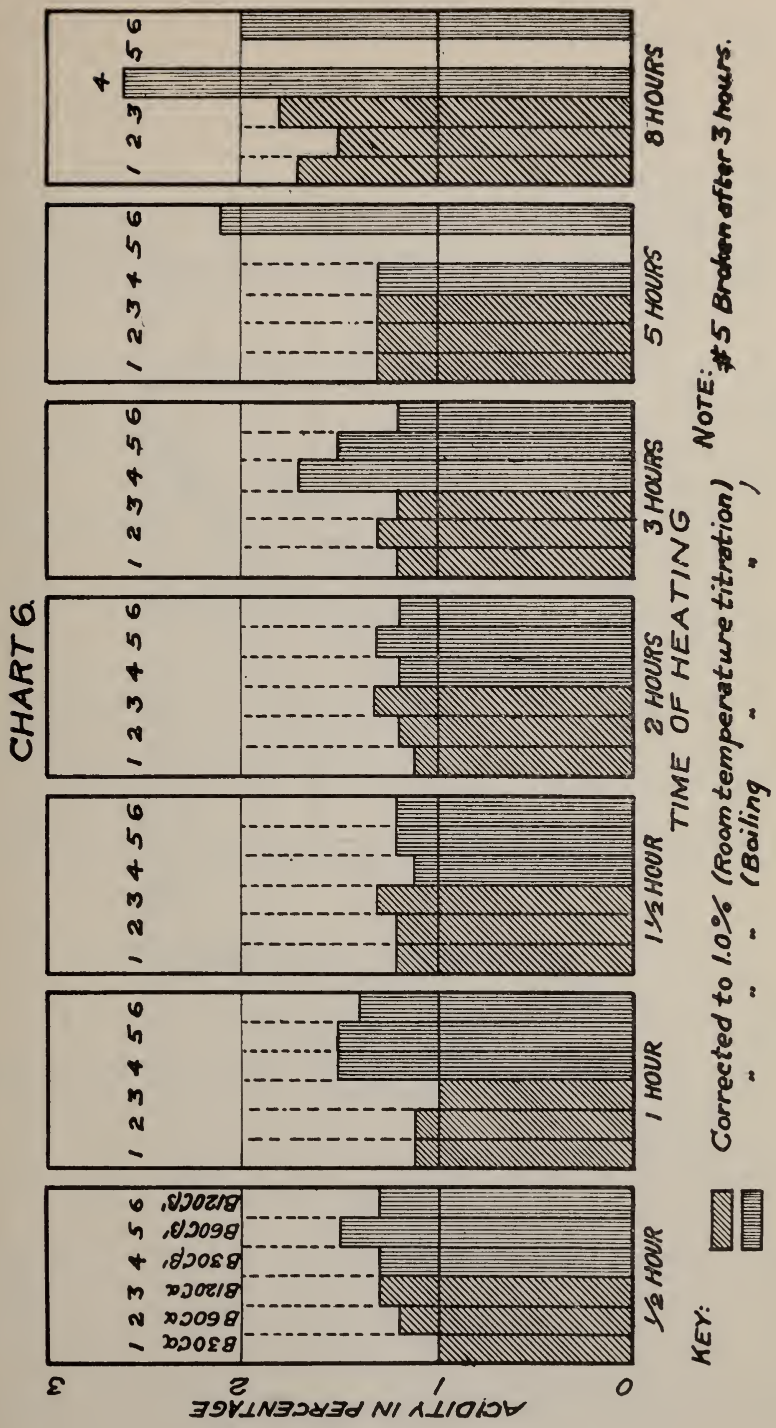
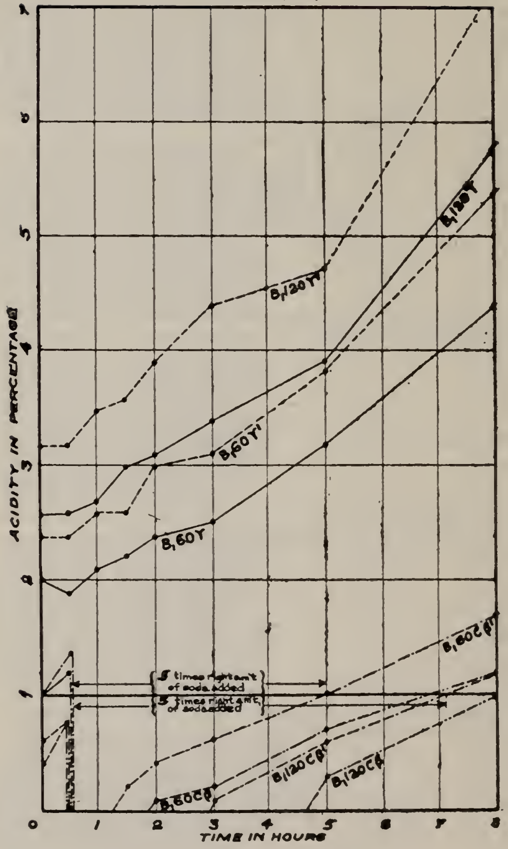


CHART 6.

ACIDITY IN PERCENTAGE
0
1
2
3
1 2 3 4 5 6
B30Cα
B60Cα
B120Cα
B30Cβ'
B60Cβ'
B120Cβ'
½ HOUR
1 HOUR
1½ HOUR
2 HOURS
3 HOURS
5 HOURS
8 HOURS
TIME OF HEATING
KEY:
▨ Corrected to 1.0% (Room temperature titration)
▤ " " " (Boiling " ")
NOTE: #5 Broken after 3 hours.

CHART 7.


ACIDITY IN PERCENTAGE
1
2
3
4
5
6
7
0
1
2
3
4
5
6
7
8
TIME IN HOURS
B,120Y'
B,60Y'
B,60Y
B,120Y
B,60Cβ'
B,120Cβ'
B,120Cβ
B,60Cβ
5 times right am't of soda added
5 times right am't of soda added

EXPERIMENTAL DATA COMPARING THE DELICACY OF DIFFERENT TESTS FOR HYDROGEN PEROXIDE IN MILK

Irwin T. Darlington

(*Reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 7, No.* 8, *page* 676. *August,* 1915)

From references on the detection of hydrogen peroxide, the reagents listed in Table I were found to be in most general use and appear to give the most delicate tests for the detection of hydrogen peroxide in milk.

A stock solution of hydrogen peroxide was made from a commercial sample containing 3.2 per cent. of hydrogen peroxide by analysis with potassium permanganate. This stock solution was made so that each c.c. contained 0.01 g. of hydrogen peroxide.

TABLE I

Reagent	Solution	Color in Presence of H_2O_2
Starch................	Dilute (water) + KI........................	Bluish green.
Paraphenylene diamine..	2 per cent (water)..........................	Blue.
Benzidine.............	4 per cent. (alcoholic) + 10 cc. milk (+ 2 or 3 drops acetic acid)........................	Blue.
Titanic acid..........	2 per cent. solution titanium hydrate in dilute H_2SO_4	Yellow.
Vanadic acid..........	2 per cent. in dilute H_2SO_4	Faint orange-red.

The milk to which the hydrogen peroxide was added was received from the city inspectors and was proven free from hydrogen peroxide by each of the above reagents.

Hydrogen peroxide was added to each 100 c.c. of milk in separate bottles to the amounts indicated in Table II, and tests made on 10 c.c. of each sample with each of the above reagents. Tests were made immediately after the samples were made up and again after standing 18 hours in a warm room.

TABLE II

Grams Per 100 cc.	Results of Tests Made Immediately After Samples were Made Up															After Standing 18 Hrs. in a Warm Room		
	15.0	9.0	3.0	1.5	0.75	0.6	0.3	0.15	0.03	0.015	0.006	0.003	0.00105	0.00075	0.00037	15.0	9.0	3.0
Starch...............	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–
Paraphenylene........	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–
Benzidine............	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–
Titanic Acid..........	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–
Vanadic Acid.........	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–

It will be seen from Table II that paraphenylene diamine and benzidine proved to give the most delicate tests for hydrogen peroxide in raw milk. The limits of these two reagents proved to be 0.00075 g. of hydrogen peroxide in 100 cc. of raw milk. No experiments were made with milk that had been previously heated to destroy bacterial action.

Practically all the hydrogen peroxide was reduced after standing 18 hours in a warm room. This confirmed previous results obtained in this laboratory when it was found that after 18 hours' standing at room temperature no reaction could be obtained from the addition of 1 g. of magnesium peroxide to 100 cc. of raw milk. Positive tests were given only when the milk contained 9 per cent. and 15 per cent. of hydrogen peroxide.

The samples containing 9 per cent. and 15 per cent. of hydrogen peroxide failed to give a positive test with any of the above reagents, except paraphenylene diamine, which gave a delicate positive test with 15 per cent. of hydrogen peroxide after standing in a warm room for 24 hours. Tests on distillates of milk treated with peroxide were negative.

A few tests were made with magnesium dioxide added as a preservative. The experiments made with this material were insufficient to afford any positive conclusions, but it appears that magnesium dioxide does not lend itself to as delicate tests as does hydrogen peroxide.

From the foregoing it is seen that paraphenylene diamine is the test of the reagents tested for the detection of small quantities of hydrogen peroxide in raw milk, and that it is probable that peroxide in amounts that would be added to milk as a preservative cannot be detected after 18 hours by these reagents.

THE VIABILITY OF THE TYPHOID BACILLUS IN SOUR CREAM

Charles Krumwiede, Jr., M. D., and W. Carey Noble

(*Reprinted from Dept. of Health Reprint Series, No.* 23, *Jan.,* 1915)

Numerous investigations have shown that typhoid bacilli quickly die in sour milk, butter-milk, and other sour milk preparations. The bacilli are killed by the acid produced, as is shown by the fact that lactic and butyric acid have been found to kill typhoid bacilli with varying rapidity, depending on their concentration.

Recently it was claimed that the Department of Health need not insist on pasteurization of sour cream, as the acidity would quickly render it safe, should typhoid bacilli be present. The following experiments were undertaken to determine whether this deduction from the results of previous observers was justified. Not only was the rate of disappearance in naturally souring cream determined, but also in sour cream under conditions where overgrowth by other bacteria was eliminated.

TABLE I

Sour Cream —27,000,000 bacteria per cc.
Sweet Cream —11,000,000 bacteria per cc.

Typhoid Suspension=1-10,000 dilution of 24-hour broth culture used
30,000 per cc.

1. 50 cc. cream+5 cc. typhoid suspension
2. 50 cc. cream+1 cc. typhoid suspension
3. 50 cc. cream+.01 cc. typhoid suspension
4. 50 cc. cream+.0001 cc. typhoid suspension

Mixtures			4/17	4/18	4/19	4/20	Total Counts 4/20	
Sour Cream	RoomTemp.	1.	90%	0.1%	Neg.	Neg.	90,000	Apparent discrepancy between few typhoid added and percentage found is due, partly, to inhibition on special plating media.
		2.	20%	0.1%	Neg.	—		
		3.	3%	0.1%	Neg.	—		
		4.	0.1%	Neg.	Neg.	—		
	IceBox.	1.	90%	3%	Neg.	Neg.	250,000	Both series, 4/20, gave practically no growth on Conradi, only very fine colonies present.
		2.	30%	0.5%	Neg.	—		
		3.	20%	0.1%	Neg.	—		
		4.	1%	0.1%	Neg.	—		
Sweet Cream	RoomTemp.	1.	98%	8%	Neg.	Neg.	80,000,000	Accurate estimates difficult because of great numbers of non-lactose-fermentingtypes
		2.	40%	5%	Neg.	—		
		3.	20%	Neg.	Neg.	—		
		4.	2%	Neg.	Neg.	—		
	IceBox.	1.	90%	2%	10%	20%	92,000,000	Sweet Cream, Icebox specimen No. 1, was taken out of the icebox April 20, and kept at room temperature. The next day it was negative.
		2.	45%	0.1%	Neg.	—		
		3.	15%	0.1%	Neg.	—		
		4.	0 1%	Neg.	Neg.	—		

By comparing the results with the different samples it seems that the inability to recover typhoid bacilli was due in part to the overgrowth by other bacteria. To determine how far this was a factor, the following tests were made:

A. To fifty c.c. of cream were added 7,000,000 typhoid bacilli. The cream was allowed to sour at room temperature and the acidity as well as the percentage of typhoid bacilli determined.

Plated, at once	Typhoid 90%	Reaction 1.02 acid[*]
Plated, after 24 hours	Typhoid 2%	Reaction 4.7% acid[*]
Plated, after 48 hours	Typhoid 0%	Reaction 6.8% acid[*]

B. Fifty c.c. was allowed to sour over night, heated in the Arnold sterilizer and typhoid bacilli added as above. The heated cream gave no growth when plated on agar.

Plated, at once	140,000 typhoid bacilli per cc.	Reaction 3.6% acid
Plated, after 24 hours	80,000 typhoid bacilli per cc.	Reaction 3.6% acid
Plated, after 48 hours	55,000 typhoid bacilli per cc.	Reaction 3.6% acid
Plated, after 96 hours	4,000 typhoid bacilli per cc.[†]	Reaction not tested
Plated, after 120 hours	0 typhoid bacilli per cc.[‡]	Reaction 7.6% acid

From these results it is evident there is a reduction in the number of typhoid bacilli when the acidity rises above 3 per cent.

Because of the contamination of the above series the tests on sterilized sour cream were repeated, using two samples of cream at different degrees of acidity. The mixtures were kept at room temperature.

Plated	7% Acid No. of Typhoid Per cc.	5.6% Acid No. of Typhoid Per cc.
At once	375,000	385,000
After 24 Hours	110,000	230,000
After 48 Hours	7,800	56,000
After 72 Hours	200	3,000
After 96 Hours	0	0

To obtain a souring cream, but at the same time to avoid the overgrowth of the typhoid bacilli on the plating medium, cream was inoculated with typhoid bacilli and B. Metchnikovii. The mixture was kept at room temperature and the souring for this reason proceeded very slowly.

VIABILITY OF TYPHOID BACILLUS IN SOUR CREAM

Days	0	2	7	8	9	10	11
Reaction	1.0%	2.2%	5%	—	—	—	10%
No. of Typhoid.	392,000	65,000,000	300,000,000	113,000,000	181,000	2,600	400

Evidently the time required to kill the bacilli is directly proportional to the number of typhoid bacilli present.

Summary.—The typhoid bacillus is gradually killed in sour cream by the acids produced, the rate of destruction being proportional to the degree of acidity and the number of typhoid bacilli present. The apparent disappearance of typhoid bacilli in sour cream where the normal flora is present is due in part to the overgrowth of the typhoid bacillus by these bacteria and the difficulty of finding by our present methods the proportionately few typhoid bacilli remaining.

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[*] Number of cc. of normal alkali needed to neutralize 100 cc. of cream, phenolphthalein being the indicator.

[†] Contamination, 2,000,000 colonies per cc. in agar. Most of the types do not develop on Conradi.

[‡] Total count, 15,000,000.

With a moderate contamination, the typhoid bacilli are killed in about four days. With a heavy contamination or where initial multiplication has taken place, a longer time may be required. For this reason a clean cream which soured slowly would be more dangerous if contaminated, as an initial multiplication of the typhoid bacilli would occur and a longer time would be required to destroy the bacilli. Whether under ordinary conditions the overgrowth by the bacteria of the cream is a factor in the death of the typhoid bacilli cannot be determined by our present methods.

A DEVICE FOR THE SUCCESSIVE DETERMINATION OF THE SOLIDS AND FAT IN MILK AND OTHER FLUIDS

Armin Seidenberg

(*Reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 7, No. 9, page* 769. *September,* 1915)

A large number of methods for the accurate gravimetric determination of fat in milk have been developed. In one class of methods of which the Rose[1]–Gottlieb[2] and the Werner-Schmidt[3] are examples, the proteids are first brought into solution by the proper reagent, after which the fat is extracted by agitation with a solvent. These methods require a considerable amount of manipulation on the part of the analyst, thus demanding a good deal of his time and attention. On the other hand, the direct extraction of the dry residue of the milk in a Soxhlet or modified Soxhlet apparatus, although the results are not obtained as quickly, consumes practically no more of the analyst's time than is required to make the necessary weighings.

In order that the solvent may act effectively on the dry milk residue, it is of advantage to have it in a finely divided state. To bring this about a number of materials have been suggested, thus: sand, pumice stone, asbestos, copper sulfate, plaster of Paris, wood pulp, absorbent paper, etc. Of all these substances the Adams[4] fat-free paper offers probably the conditions that are most favorable to the complete extraction of the fat since the pores of the paper absorb the water in the milk very readily, leaving the fat on the surface where it is most exposed to the action of the ether. However, there is always some difficulty in transferring a weighed quantity of the milk on to the paper; a weighing flask being desirable for most accurate work; also a slight amount of extractible matter remains in the paper commonly supplied to analytical laboratories.

When a material is chosen that is not itself hygroscopic, the possibility is presented of making successive determinations of the total solids and fat on the same original weighing. In this case the fat can also be determined by difference and the solids-not-fat, when these are desired, can be directly weighed instead of being computed from the results of two different determinations. For the determination of total solids and fat (by difference) and of solids-not-fat, only four weighings are then necessary instead of seven, where separate quantities are weighed out for solids and for fat. The Adams fat-free paper is of course not adapted to the determination of fat by difference since it is intensely hygroscopic. The material that has gained most favor for this purpose is asbestos, first proposed by Babcock for the determination of total solids and developed by Richmond[5] and also by MacFarlane[6] for the determination of fat by difference. As adopted by the Association of Official Agricultural Chemists,[7] 1.5 to 2.5 grams of asbestos are loosely packed into a perforated sheet-metal cylinder 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a perforated disc of the same material. The total solids are first determined and then the fat either by difference or by weighing directly. The asbestos must be prepared first. Freshly ignited woolly asbestos is used from which all grit and small particles must be carefully removed.

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[1] Zeitschrift angew. Chem., 1888, p. 100; Milch. Ztg., 18 (1888), 264.

[2] Landw. Vers. Sta., 40 (1892), 6

[3] Zeitschr. f. analyt. Chemie, 27 (1888), 464.

[4] Analyst, 10 (1885), 46.

[5] Analyst, 17 (1892), 225.

[6] *Ibid.*, 18 (1893), 85.

[7] U. S. Dept. of Agr., Bureau of Chemistry, Bull. 107, 117.

DESCRIPTION

Whatever medium is chosen to hold the milk, its function is to permit the ready access (1) of heat at a uniform temperature and (2) of the solvent used for extraction. This is accomplished by using a material that distributes the milk solids in a finely divided state over as large a surface as is possible and which, while permitting ready access to both the solvent and heat, is acted upon by neither of these. It was with these considerations in mind that it occurred to the writer that a fine meshed wire gauze seemed to meet all the conditions enumerated, while at the same time it offered some advantages in respect to simplicity and reliability not possessed by any of the substances so far employed. The particular modification finally adopted by the writer is as follows: An oxidized copper wire gauze of 40 or 60 mesh is used, ridged (or pleated) laterally with about 18 to 20 ridges. The two long sides of the gauze are bent up for 2 mm. in such a way as to close the opening of the grooves formed by the ridges. The dimensions of the gauze are 7 × 10 cm., the ridges being about 2 mm. high and about 2 mm. apart. The gauze is placed upon two ridges 2 mm. high running the length of a dish made of heavy tinfoil on whose surface are numerous cross-sectional indentations. The dimensions of the dish are 7 × 10½ cm.; three of the sides are 4 mm. high and the fourth (a long side) is 8 mm. high. The sides are not joined at the corners.

In making determinations of solids and fat the wire gauze and dish are first weighed accurately and then 4 or 5 grams (or other weighed quantity) of milk are evenly distributed drop by drop by means of a pipette over the entire gauze. In some respects the most satisfactory amount to use is 4 grams. If too great an amount of milk has been added to any one portion of the gauze it can readily be withdrawn with the pipette and redistributed. The milk is then dried to a constant weight in an air bath at 100° C. and the device again weighed to find total solids. Particular attention must be given to see that the liquid is well distributed and that the gauze is held level throughout and not subjected to any jolting. For the determination of fat the gauze is rolled into a conical form along with the dish, the high side and the ridges of the dish being first flattened out. After the gauze and dish have been rolled up the high side at one end is folded up, the other end being left open. The device is now introduced into a Soxhlet or other extraction apparatus and subjected to the action of the extracting medium. It is then dried again to constant weight, the loss in weight being fat.

DISCUSSION

An oxidized or a well-flamed copper gauze was used, because it was found that with an unoxidized gauze the milk tended to gather in drops, but when the gauze was oxidized the milk spread out over the surface of the gauze and entered the interstices of the meshes without, however, going through even when the grooves formed by the ridges were well filled with liquid. The bent up edges of the gauze close the grooves at each end and prevent the milk from flowing out there. However, should a few drops go through the meshes, they would be caught on the containing dish. The amount would be so small that it would be well spread out and not affect the results. The gauze is placed upon two ridges running lengthwise on the bottom of the containing dish. The object of these ridges is to prevent direct contact between the gauze and the bottom of the dish as in this case some of the milk is at times drawn into the dish by capillary attraction. After drying, the solid matter will be found to be distributed quite uniformly over the surface of the gauze, and will adhere firmly to it as though forming an integral part of it. There is no evidence of cracking as when milk is evaporated in a dish; nor is there any charring as would be shown by a yellowish or brownish discoloration. When the device is placed in the extraction apparatus, the end closed by the one long side is placed toward the bottom. After using once it is best to discard the containing dish; the wire gauze, however, may in some cases be used again. To do this the milk solids are first burned off, the ash washed out with water and the gauze then dried thoroughly in the flame. It would seem that by repeating this process the wire gauze could be used indefinitely, but the writer has found that after the third and at times after the second use a very few particles of the oxide become loose and are washed out by the ether. This danger does not exist at all with a new gauze oxidized the first time and usually not with one used but twice. However, for most accurate work it would seem best to use only a new gauze. For the determination of total solids alone the gauze can be used repeatedly after burning off the matter from the previous determination. The wire gauze makes certain the wide and uniform distribution of the milk solids over a large horizontal surface, while in the case of material consisting of small particles or fibers held in a vertical cylinder or dish, the liquid may, if the packing is imperfect, gather largely in one part of the apparatus without the observer knowing it. The gauze, while permitting the free passage of the extracting medium, is not in any way affected by this, either chemically or when a newly oxidized gauze is used, by the washing out of any loose particles.

For the purpose of this investigation the wire gauze and dishes used were made in the laboratory. For the milk, a 60 mesh copper wire gauze was used, the same that is employed in combustion work; for cream a 40 mesh copper gauze is more desirable. Where a large number of determinations of this character are to be made the device can be turned out, ready for use, by a manufacturing concern. As opposed to the asbestos and other material so far suggested, no preliminary work of preparation is then necessary; *i. e.,* no other than is required before using a lead or platinum dish in determining total solids. Instead of using a Soxhlet extraction apparatus the writer found it practicable to use a Landsiedl extraction apparatus with a siphoning tube that held four gauzes rolled into the cylindrical form. The Landsiedl extraction apparatus is in many respects similar to the Soxhlet extraction apparatus except that the siphoning tube instead of being held in a special adapter placed between the flask and the condenser, is suspended in the enlarged neck of the flask which is directly connected to the condenser. The height of the siphoning tube should be slightly greater than the height of the wire gauze used and the neck of the flask should be adapted to this. An apparatus with a wider siphoning tube capable of holding an even larger number of gauzes might well be used. The fat, of course, can be determined only by difference. The saving of time and trouble of manipulation in using one Landsiedl apparatus in place of four or more Soxhlets is evident.

DETERMINATIONS

In order to ascertain the reliability and accuracy attainable by using the wire gauze as described, the writer made a large number of determinations, under varying conditions, of both the fat and the solids of milk. First of all, tests were made to determine to what extent, if any, the device was affected either by heat at the temperatures employed or by the extracting medium. No change of weight that could be due to either of these two causes was noticeable. The gauze and dish were heated in the air bath at temperatures from 100° C. to 110° C. for prolonged periods of time without there being any significant gain or loss of weight. The same can be said when 5 c. c. of distilled water were added and heating carried on under the same conditions. There is therefore no danger of oxidation of the copper when heated under the conditions described. The ether also had no perceptible effect on the weight of the gauze and dish.

The determinations made with the wire gauze for total solids were checked by making determinations under exactly the same conditions in lead dishes, of 5.5 cm. diameter and which have always been used in this laboratory for this purpose. The fat determinations were checked with the Adams fat-free paper as supplied by the firm of Schleicher and Schüll. The extractions were made with ether in an Adams modification of the Soxhlet apparatus. On evaporating the ether alone, spontaneously, no residue remained. However, when the ether was boiled with a reflux condenser for about 4 hrs. it left a slight residue; on prolonged drying this was considerably reduced. The writer found that the fat-free paper used by him gave an extract after 4 hrs. treatment with ether, amounting to from 0.0041 to 0.0062 g., an average of 0.0051 g. Siegfeld[1] states that he found the Schleicher and Schüll fat-free paper to give an extract amounting to from 0.006 to 0.009 g.; in some cases to 0.012 g. Leffmann[2] and some others, however, give a much lower figure, namely 0.001 to 0.002 g. When 5 grams of milk are used originally, and the results expressed in percentages, an error of from 0.120 to 0.180 per cent. would be introduced on the basis of Siegfeld's determinations and of 0.082 to 0.124 per cent. on the basis of the writer's determinations. This error can be corrected either by subjecting the paper to several hours treatment with ether previous to using, or by subtracting in each case the amount due to the extract from the paper. In the determination of fat made by the writer with the Adams fat-free paper a uniform amount of 0.10 per cent. was subtracted from all the results obtained.

Total Solids—Determinations for total solids were made on 39 different samples of milk; in most cases two series of determinations were made in a lead dish and two on a wire gauze; weighings were then made after the drying of both the lead dish and the wire gauze containing the same samples under similar conditions at 100° C. at intervals of 2 to 4 hours. In some cases the solids were subjected to temperatures varying from 60° C. to 80° C. for from 16 to 18 hours. In all, 461 weighings were made on the 39 samples. The drying was carried on entirely in an electric oven automatically regulated to maintain a temperature of within 2° to 3° of 100° C. Milk solids lose weight almost continuously at 100° C.; when, however, according to Blythe[3] the loss for 5 grams of milk is at the rate of 1 milligram or 0.02 per cent. or less per hour, the residue can be considered to have reached constant weight. The rates of evaporation in the various cases, even where the same device was used, were not uniform. Where the drying is continuous, constant weight is usually reached after about 5 hours heating at 100° C. in an air bath. When drying is interrupted, however, a longer time is necessary. After constant weight has been reached the rate of loss of weight is almost always the same, where the solids are dried in the same device. On several occasions there was a slight gain instead of loss of weight on heating the solids both on the wire gauze and in the lead dish. The cause to which this is due is not entirely clear. It seems, however, in most cases to be due to a slight oxidation of the fat in the milk, rather than to absorption of moisture or any other cause. The amount at which the solids in the lead dish reached constant weight was almost in every case lower than that at which constant weight was reached when the wire gauze was used. Of the 39 samples on which determinations were made for total solids there were 35 in which the results obtained with the wire gauze were higher than those obtained with the lead dish by an average difference of 0.07 per cent. In no case was the difference as much as 0.2 per cent and in only 5 was it between 0.15 per cent. and 0.20 per cent. In only 4 out of the 39 samples were the solids obtained by using the wire gauze equal to or lower than those obtained when using a lead dish. In these cases the differences were much less than in the former case, namely, on an average of 0.04 per cent.—the greatest difference being 0.052 per cent. Taking the averages of all the determinations at constant weight made on the 39 samples—*i. e.,* 83 determinations at constant weight with the wire gauze and 63 check determinations at constant weight with the lead dish—it was found that the average of all the results obtained with the wire gauze was 0.06 per cent. above the average of the results obtained with the lead dish. In a number of instances heating was continued after constant weight had been reached. While the solids lost weight in both cases, the rate at which the solids in the lead dish lost weight was much greater than that at which the solids on the wire gauze lost weight. Thus in 3 of the samples the losses of weight of the solids in the lead dish due to heating for a prolonged period of time after constant weight had been reached amounted to 0.25 per cent, 0.19 per cent and 0.08 per cent, respectively, or an average of 0.17 per cent. During the same periods of time the drops in weight from constant weight of the corresponding solids on the wire gauze were 0.12 per cent., 0.03 per cent. and 0.02 per cent, respectively, or an average of 0.06 per cent. It will thus be seen that on the wire gauze the milk solids reached constant weight at an average of 0.06 per cent. above the point at which constant weight is reached by the solids in the lead dish, and that this weight changes considerably less during a continued period of heating than is the case when a lead dish is used. This, of course, substantiates the statement made as to the uniform heating and resultant absence of disintegration of the solids on the wire gauze.

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[1] Zeitschr. f. Untersuch. d. Nahrungs u. Genussmittel, 6 (1903). 259.

[2] "Anal. of Milk and Food Products," Ed. 1905, p. 12.

[3] "Foods: Their Composition and Analysis," Ed. 1903, p. 215.

Fat—Fat determinations were made on 11 samples of milk. On each sample two determinations were made by the Adams method and two or more with the wire gauze, the fat in the latter case being determined in each instance both by direct weight in the flask and by difference on the wire gauze. Practically all the fat is extracted after 2 hours extraction; however, extraction was carried on in every case for 4 hours. The Adams modification of the Soxhlet apparatus used made from 15 to 20 extractions per hour. The fat flasks were allowed to stand in the air 10 to 15 minutes before weighing since when perfectly dry they gain weight very rapidly. This is similar to the procedure recommended by Siegfeld[1] who suggests, however, that the flasks be wiped off with a fine silk cloth before weighing. This latter was omitted by the writer as of rather dubious value. Siegfeld states that the errors of weighing the fat flasks amounted to from 0.008 to 0.012 g., or where 5 grams of milk are used to from 0.160 to 0.240 per cent.

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[1] "Zeitschr. f. Untersuch. d. Nahrungs u. Genussmittel, 6 (1903) 259."

However, where the procedure described is pursued, these errors are considerably reduced. After the fat has all been extracted, 2½ to 3 hours drying at 100° C. are sufficient to bring the solids-not-fat to constant weight. Since the loss in weight after the total solids or solids-not-fat have reached constant weight is less than 0.02 per cent. per hour, the determination of the fat by difference can be secured with a high degree of accuracy. This is particularly true in view of the fact that the weighings can be made before an appreciable amount of moisture is absorbed from the air, thus presenting an advantage over the determination of fat involving the weighing of fat flasks. The extent to which the total solids and the solids-not-fat are hygroscopic amounts according to the writer's determinations to no more than a gain of 0.05 per cent. in 10 minutes. After correction has been made for the extractable matter from the Adams fat-free paper the results obtained by this method and where the fat is determined by means of the wire gauze, both by difference and by direct weight, are in practical agreement. The average of all the corrected results by the Adams method, and the corresponding averages obtained by the wire gauze method, both by difference and by direct weight, are respectively 2.854, 2.868, and 2.859 per cent.

Very satisfactory results were also obtained in making determinations of fat and total solids of creams, skimmed milks, and of homogenized milks and creams. For sweetened condensed milks it was found that unless a very dilute solution was used, the sugar present prevented, somewhat, the complete extraction of the fat. However, for the determination of total solids of sweetened condensed milks the wire gauze may be employed to particular advantage. The charring of the large amount of sugar present in condensed milk introduces a serious error when a dish is used for the determination of total solids. It makes the attainment of constant weight at 100° C. practically impossible. When 5 grams of a 40 per cent. solution of sweetened condensed milk, which is about twice to three times the consistency of ordinary milk, are evaporated on a wire gauze, constant weight, as previously defined, is obtained in the usual time and there is no discoloration of the residue to show charring. Richmond[1] found that the Adams method gave low results when used for determining the fat in homogenized milk or cream. This he ascribed to the fact that the subdivided fat globules of the homogenized milk were drawn into the cores of the Adams fat-free paper and there, being surrounded by the fibres of the paper, did not come into effective contact with the extracting medium. The determinations made by the writer would seem to confirm these conclusions. The wire gauze is not open to these objections and the results obtained by it in determining the fat in homogenized milk and cream were higher than those obtained when the Adams method was employed.

Conclusion

Besides milk and milk products it would seem as though the wire gauze, by using an appropriate modification, is well adapted for the determination of the solid matter of a large number of other substances such as: glues, varnishes, shellacs, some oils, tanning materials, etc.; syrups, flavoring extracts and other saccharin products; and for the extracts of vinegars, beers, wines and distilled liquors. Thus for liquors and other thin fluids an 80 mesh wire gauze was found suitable. The device has been used lately with very satisfactory results by J. Wiener of this laboratory in the determination of the solid matter in medicinal preparations containing considerable amounts of sugar and glycerine. The solids reached constant weight without charring at a percentage in quite close agreement with that found from the summation of the individual constituents contained in the preparation. When a dish was used there was always charring, even though the results were considerably too high, and it was impossible to establish at what point constant weight had been reached. In conclusion it may be said that the device gives particularly reliable results in the determination of total solids of milk and of other substances that tend to char easily. The results for fat and for solids-not-fat of milk are also in every way accurate and reliable and compare favorably in this respect with those obtained by other methods, no corrections or blank determinations being necessary. While care is demanded in the manipulation of the device a means is provided that can be kept ready for use in the laboratory and that permits the successive determination of solids and fat of milk and the direct determination of the solids-not-fat, thus effecting a very considerable saving of time on the part of the analyst.

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[1] Analyst, 31 (1906), 218.

THE SPINAL FLUID IN POLIOMYELITIS AND ITS DIFFERENTIATION FROM FLUIDS OF OTHER INFECTIONS*

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A review of the literature reveals comparatively few extensive studies of the cerebrospinal fluid in acute poliomyelitis. This fact is due perhaps to the lack of opportunities for such studies, or to the failure to appreciate the value of the changes in such fluids.

Wickman[1] in his exhaustive monograph on poliomyelitis pays only scant attention to the subject of the fluid in this disease. Römer and Joseph[2], too, slight the value of examination of fluids in their discussion of diagnosis. They have been particularly interested in the attempt to demonstrate specific substances in the fluid and serum by complement fixation. In this attempt they have failed, as also have Wollstein[3] and Gay and Lucas.[4]

Plaüt,[5] Rehm and Schottmüller devote a page to the cerebrospinal fluid of poliomyelitis in their book on cerebrospinal fluids. They say that cerebrospinal fluids in poliomyelitis contain no globulin and that the average cell count is 15 per cubic millimeter.

Forbes[6] reports the examination of 33 cerebrospinal fluids of which 8 were bloody, due no doubt to accident in technic. Of these, 24 contained albumin in varying amounts. Six showed a heavy precipitate. Cytologic examination showed increase of cells in all but 6, twenty-one fluids having only a slight excess. Very few polymorphonuclears were found, small mononuclears being the most common type. He found that the cell reaction was in direct proportion to the albumin content. All of these cerebrospinal fluids reduced Fehling's solution normally.

Morse[7] reported thirteen examinations of cerebrospinal fluids in seven cases and found pathologic changes in all. All fluids were clear, with one exception, and this was opalescent. The small mononuclear cell was the predominating type of cell. He does not find any aid from the examination of the spinal fluid in differentiating poliomyelitis in preparalytic or abortive form from tuberculous meningitis.

Gay and Lucas[4] have made an interesting cytologic study of spinal fluid from 6 cases of monkey poliomyelitis, noting changes in all stages of the disease. In the incubation period the cells were increased from 100 to 300 per cubic millimeter. They were largely of the large mononuclear type with some polymorphonuclears. In the prodromal period the count went up to 1,000 per cubic millimeter. In this stage polymorphonuclears were increased, in some cases, up to 60 per cent. In the early acute stage, the increase of cells was still very great but the lymphocytes were the predominating cells. At the end of a week or ten days, there were very few cells present, mostly large mononuclears and a few polymorphonuclears. They also report on thirteen spinal fluids from 4 human cases, all of which showed the large mononuclear cell present in varying numbers.

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* Read before the Joint Meeting of Brooklyn Pediatric and Neurologic Societies, May 26, 1915.

[1] Wickman, Ivan: Jour. Nerv. and Ment. Dis., Monograph 16, 1913.

[2] Römer and Joseph: München. med. Wchnschr., 1910, vii, 568.

[3] Wollstein, Martha: Jour. Exper. Med., 1908, p. 476.

[4] Gay and Lucas: Arch. Int. Med., 1910, vi, 330.

[5] Plaüt, Rehm and Schotmüller: Leitfaden zur Untersuchung der Zerebrospinal flussigkeit, Gustav Fischer, 1913.

[6] Forbes, J. G.: Lancet. London, Nov. 18, 1911.

[7] Morse, J. L.: Arch. Pediat., 1911, xxviii, No. 3.

Probably the best and most comprehensive study of the cerebrospinal fluid in poliomyelitis in the American literature is that of Peabody, Draper and Dochez.[8] They tabulate and analyze the findings in 233 spinal fluids from 69 patients. The conditions of their study were such as to permit the following up of the changes in the fluid from week to week, and they have compiled the results admirably. All but two of the cases presented abnormal changes either in cell content or chemical constituents, most frequently in both. The predominating type of cell was the smaller mononuclear. In some cases, early in the disease, polymorphonuclears were present up to 80 to 90 per cent. of the total count. Mention is made of large phagocytic cells, containing vacuoles and broken down cells as being present in some cases. A grouping of the spinal fluids into two distinct types is made, the one with a high cell count and a low globulin content; the other, with a comparatively low cell count and a high globulin content. The former is found most frequently in the early part, and the latter in the later part of the acute stage.

Four preparalytic and two abortive cases are included in this report and are of particular interest inasmuch as these are the types of cases that present the greatest difficulty in diagnosis. Two of these patients that developed paralyses showed a high cell count. Three others showed only a moderate increase in cells. Of these two developed paralyses and one was an abortive case. The remaining one, an abortive case, presented a normal spinal fluid on first examination, but the cells were found increased in later specimens. They urge that though the cerebrospinal fluid of poliomyelitis does not present a specific picture, it is of the utmost value as an aid, particularly when considered in conjunction with the clinical aspects of the case, in clearing up the diagnosis in the abortive type and in the preparalytic stage.

The material for the present report was taken from the records of the meningitis department of the New York City Board of Health and covers a period of four years. It consists of the examination of forty-seven cerebrospinal fluids from twenty-nine patients, twenty-seven of which were seen by members of the meningitis staff in consultation with physicians throughout the city. Comparatively few of these patients were seen more than once, and we were, therefore, unable to observe the changes in the various stages of the disease that the Rockefeller Institute workers so ably described. However, this work compensated in the great interest produced by the necessity of arriving at a correct diagnosis through the examination of the spinal fluids, as the majority of the cases were of the meningitic and encephalitic varieties.

Of the 47 fluids, 2 were contaminated by blood and 2 were otherwise unreliable. Of the remaining 43, 10 were from cases diagnosed as myelitis, 21 from encephalitic, 6 from myeloencephalitic, and 6 from abortive cases. Of the 10 myelitic cases, 5 fluids were examined in the preparalytic stage and 5 in the postparalytic stage. Of the 21 encephalitic fluids, 17 were from patients without paralysis, many of which properly belong to the meningitic type, and 4 were from cases with involvement of the cranial nerves. The 6 fluids of the myeloencephalitic group were obtained from cases that presented involvement of both the cranial nerve nuclei and anterior horn cells. The abortive cases showed no signs of paralysis. We feel quite secure in the diagnosis of the cases not showing paralysis, by the clinical characters and outcome of the cases, and in fatal cases, by the negative results after inoculation into two guinea-pigs.

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[8] Peabody, Draper and Dochez: Monograph 4, Jour. Exper. Med., June 24, 1912.

Of the 43 fluids examined, 28 were obtained in the first week of the disease; 8 in the second week, 5 in the third week and 2 in the fourth week.

The routine of our examination consists of a description of the amount and gross appearance of the fluid; an estimation of the cell contents, as to whether there is no increase, a moderate increase or a great increase. This was accomplished by centrifuging 10 to 15 c.c. of the fluid at high speed for one-half hour and examining three or four platinum loopfuls of the sediment spread on a slide and stained; a rough differential count of the types of cells present; also making note of unusual types of cells. The fluid is then examined bacteriologically on smear and in culture. If smear is negative for organisms, 5 to 10 c. c. of the fluid is inoculated into each of two guinea-pigs. The chemical examination consists of the estimation of the albumin and globulin content and the reduction of Fehling's solution. The albumin and globulin content is graded in + signs, beginning with ± and ascending to 4 +, depending on quantity of precipitate. A ± is considered as that very faint haze present in normal fluids, and 4 +, the maximum, represents the very heavy precipitate obtained most commonly in purulent fluids. The reduction of Fehling's solution is graded, negative when there is no red precipitate at the end of 15 minutes; 1 ± when there is a slight precipitate at the end of that time; 2 + when there is moderate precipitate, and 3 +, the maximum, when there is an immediate and heavy reduction. This latter reaction is encountered in practically all normal fluids.

The quantity of fluid withdrawn varied from 5 to 100 c.c. We usually withdraw as much as will readily come, minimizing the suddenness of the withdrawal as much as possible. Practically all the fluids that are much in excess of normal come out under varying degrees of pressure, though no direct method of measuring same was used. Thirty-eight fluids were clear; one slightly turbid; one opalescent; three yellowish in color but clear. One of the latter exhibited spontaneous coagulation, the so-called syndrome of Froin.[9]

CYTOLOGY

Of the 43 fluids, 15 showed a great increase; 24 a moderate increase and 4 no increase in cell content. Of the latter, one was from a paralytic case, fifteenth day of disease, with a 4 + albumin and a 3 + globulin reaction; another from a paralytic case, sixth day of disease, with no increase in albumin or globulin; one from an encephalitic or meningitic case, third day of disease, with a 1 + albumin and globulin reaction.

The small mononuclear cell or lymphocyte predominates in all but five fluids, viz., 88 per cent., in proportions varying from 60 to 100 per cent. These five show 95 per cent., 85 per cent., 75 per cent., 70 per cent. and 60 per cent. polynuclears, respectively. Of these, three were withdrawn on the third, fourth and fifth day of the disease. Of the two remaining, the date of withdrawal is unknown. Eight other fluids in which the small mononuclear predominates show a polynuclear content of from 15 to 40 per cent.

A cell that has been increasingly attracting our attention is the large mononuclear or perhaps endothelial cell. Many of them contain larger and smaller deep staining granules in their rather generous proportion of cytoplasm. This type is a rather rare find in fluids of tuberculous meningitis, in which fluids the cells are practically all of the very small mononuclear variety, with a hardly discernible rim of cytoplasm around the nucleus. We have also seen cells with polyform nuclei, described by Flexner and Lewis[10] as being present in a fluid of experimental poliomyelitis in the incubation and prodromal periods. When we find the large mononuclears in appreciable numbers in a smear from a clear fluid, we think of poliomyelitis. While we do not consider this type of cell pathognomonic of poliomyelitis, yet their presence warrants a strong suspicion of poliomyelitis.

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[9] Froin and Foy: Gaz. d. hôp., November, 1908.
[10] Flexner and Lewis: Jour. Am. Med. Assn., 1910, liv, 1140.

As 65 per cent. of our fluids are from patients in the first week of the disease, we were unable to trace any relationship between cell content and stage of disease. There was no increase in cells in 3 fluids of the first week and in one of the second week; a moderate increase in 14 of the first week; 3 of the second week; in 1 of the third week. There was a great increase in 7 of first week; in 3 of second week; 2 of third week and in 1 of fourth week.

Bacteriology and Animal Inoculation

Examination of smear for organisms, particularly the tubercle bacillus, has been uniformly negative. Cultures have been sterile except for an occasional evident contamination. Animals inoculated with 5 to 10 c.c. of the fluid have, after 4 weeks, borne the injection of 1 c.c. of concentrated crude tuberculin without lethal effect.

Chemistry

All but three fluids showed increase in the albumin and globulin content. These three were from cases in the first week of the disease.

Our findings, on the whole, show a higher albumin and globulin content in the encephalitic cases than in those of the myelitic type. This is true particularly of the nonparalytic encephalitic variety, in which the meninges are no doubt more actively involved in the inflammatory process. Of the 17 fluids in this nonparalytic encephalitic group, four show 4 + albumin and globulin; one 3 +, seven 2 +, four 1 + and one negative albumin and globulin. Of the four paralytic encephalitic fluids three showed 1 + albumin and globulin and one 3 + albumin and globulin. Of 16 myelitic fluids only one exhibited a 4 + albumin and globulin. This was a heavy yellow fluid that coagulated spontaneously. Of the remaining 15, two had 2 + albumin-globulin; twelve 1 + and one negative albumin-globulin. The six fluids from abortive myelitic cases all showed a 1 + albumin-globulin content. The findings of increased albumin and globulin content in the encephalitic type of fluid approach those encountered in tuberculous fluids, though 4 + albumin and globulin are comparatively fewer in the latter type of fluid. This tends to increase the difficulty of differential diagnosis.

TABLE 1

Albumin-Globulin Content of Cerebrospinal Fluid in Different Types of Poliomyelitis

Type	Albumin-Globulin					Total
	–	+	++	+++	++++	
Nonparalytic encephalitic.	1	4	7	1	4	17
Paralytic encephalitic....	0	3	0	1	0	4
Myelitic................	1	12	2	0	1	16
Abortive................	0	6	0	0	0	6

TABLE 2

RELATION OF ALBUMIN-GLOBULIN TO DURATION OF DISEASE

Duration Disease	Relation of Albumin-Globulin to Duration of Disease					Total
	–	+	++	+++	++++	
First week	2	18	7	1	2	30
Second week	1	3	2	1	1	8
Third week	0	2	2	1	0	5
Fourth week	0	1	0	0	1	2

We could find no definite relationship between the albumin-globulin content and duration of the disease. However, our findings do show that of 21 fluids that gave a 1 + albumin-globulin, 80 per cent. were from cases under 8 days' duration, whereas of 18 fluids that gave 2 + or better, only 40 per cent. were from cases under 8 days' duration; while 3 fluids that had no increase in these contents were respectively of 3, 6 and 9 days' duration. From these figures one might infer, not too definitely to be sure, that albumin and globulin are on the increase in the later stages of the acute disease. This is in harmony with the findings of the Rockefeller Institute workers. They also find that there is a gradual diminution in the globulin content in the more chronic stage of the disease.

RELATION OF CELL CONTENT TO ALBUMIN-GLOBULIN CONTENT

Of the four fluids showing no increase in cells, one, a yellow fluid, shows a 4 +, one a 1 + and 2 no albumin-globulin. Of 22 fluids showing a moderate increase in cells, one shows a 4 +, one a 3 +, five 2 + and fifteen 1 + albumin-globulin. Of seventeen with a great increase in cells three show 4 +, three 3 +, three 2 + and eight 1 + albumin-globulin. From these findings one could infer that the cell content parallels that of albumin and globulin. These figures agree with those of Forbes but differ from those of the Rockefeller Institute workers, who found two types of fluid, the one with high cell and low globulin in the early part of the acute stage, and the other a low cell and a high globulin in the later part of the acute stage. Our report deals largely with fluids from encephalitic or meningitic cases, which may account for the discrepancy.

Interesting to note is the fact that all three of our yellow fluids gave a 4 + albumin-globulin test, and that two of them that were tested with reduction of Fehling's solution gave only a very slight reduction.

TABLE 3

RELATION OF CELL CONTENT TO ALBUMIN-GLOBULIN

Cell Increase	Relation of Cell Content to Albumin-Globulin					Total
	–	+	++	+++	++++	
None	2	1	0	0	1	4
Moderate	0	15	5	1	1	22
Great	0	8	3	3	3	17

Reduction of Fehling's Solution

Only fifteen of our fluids were subjected to the test of reducing Fehling's solution. All of them reduced, but not in equal degree. Twelve gave a 3 + or maximum reduction and three a 1 + or minimum reduction. The relationship existing between the reduction of Fehling's and the albumin-globulin content is quite definite. The three fluids which show a 1 + Fehling's, show a 4 + albumin globulin, whereas of the twelve that show a 3 + Fehling's, none exceed 2 + and most of them show a 1 + albumin-globulin test. This relationship, in our experience, is common to all pathologic fluids. The presence of the reducing substance in varying degree, or its total absence, is the most important of chemical reactions of spinal fluids from the viewpoint of prognosis. Thus we are able to say of a case of meningococcic meningitis before the fluid is free of organisms, that the inflammatory process is receding, by noting the return of the reducing substance. Vice versa, we regard unfavorably a purulent fluid that clears up without the return of the reducing substance. In tuberculous fluids, its presence or absence indicates the extent and severity of the inflammatory process. A fluid that fails to reduce indicates an extensive and severe pathologic change.

Differentiation of Clear Fluids

The clear fluids besides those of poliomyelitis that we meet with in our work are obtained from cases of meningism accompanying pneumonia, the acute infectious diseases, and some cases of gastro-enteritis; from cases of tuberculous meningitis and from an occasional case of epidemic meningitis in which the patient is recovering. We have met with one opalescent fluid from an atypical case of rabies that strongly resembled polioencephalitis. This fluid presented a high polynuclear count and no organisms in smear and culture, comparatively low albumin-globulin and good reduction of Fehling's solution. We called it polioencephalitis until animal inoculation of the brain material proved it to be rabies.

We experience no difficulty in determining a fluid from a case of meningism, as it uniformly, in our experience, shows no increase in cells, no albumin-globulin, and a prompt and heavy reduction of Fehling's solution. Lucas[11] includes this type of fluid as presenting a cell picture similar to that found in encephalitis, poliomyelitis and tuberculous meningitis. With this we do not agree, as in none of our meningism fluids have we found an increase in cells.

The spinal fluids of patients recovering from epidemic meningitis present considerable difficulty in differentiation. They are, however, distinctly more turbid than the poliomyelitis fluids; there is a persistence of a high polynuclear content; high albumin-globulin test. These findings in conjunction with the clinical features of the case tend to clear the diagnosis.

The fluid that presents the greatest difficulty, and this is many times insurmountable, is that of tuberculous meningitis in which the tubercle bacillus has not been found. In our last 43 tuberculous fluids the organism was found 23 times, or 53 per cent. All but one of these fluids were clear. All had a preponderance of the small mononuclear cell, 85 per cent. to 100 per cent. of the total count. Comparatively few presented the large mononuclear cell, and when present, only in small numbers. Five had 1 +, eighteen 2 +, sixteen 3 + and four 4 + albumin-globulin. The behavior towards Fehling's solution is quite different from that of poliomyelitis fluids.

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[11] Lucas: Am. Jour. Dis. Child., 1911, i, 230.

TABLE 4.

SHOWING ALBUMIN-GLOBULIN CONTENT AND REDUCTION OF FEHLING'S SOLUTION.

	Albumin-Globulin						Reduction of Fehling's Sol.				
	—	+	++	+++	++++	Total	—	+	++	+++	Total
Poliomyelitis........	2	25	9	2	5	43	0	3	0	12	15
Tb. Meningitis......	0	5	18	16	4	43	12	21	6	4	43

Twelve produced no reduction; 21 only a very slight reduction, 6 a 2 + and 4 a 3 + reduction. It is to be noted also that the albumin-globulin content is higher in the great majority of tuberculous fluids. Approaching these in this respect are the fluids of the encephalitic group. Hence, a clear fluid, free from tubercle bacillus, with a high small mononuclear count, comparatively high albumin-globulin content and low in reducing substance, is more apt to be tuberculous than poliomyelitic.

A very important point in determining the diagnosis of a case with the fluid findings described as being present in either poliomyelitis or tuberculous meningitis is to be had in the history of the case. If there is an acute onset with high fever, 103 to 104, in a previously healthy person, we do not hesitate to make a tentative diagnosis of poliomyelitis. Sudden onset in tuberculous meningitis is an exceedingly rare occurrence. An excellent table of differential points in the examination of spinal fluids has been furnished by Du Bois and Neal.[12]

As to the value of lumbar puncture and examination of the spinal fluid, too much cannot be said in its favor. It can be made the means of clearing up the diagnosis in puzzling cases presenting meningeal or cerebral symptoms. Peabody, Draper and Dochez,[8] Lucas[11] and Frissel[13] present evidence of definite aid in 18 cases of poliomyelitis in the preparalytic stage. The technic of lumbar puncture is simple and safe and the examination of the fluid is not complex.

CONCLUSIONS

1. The cerebrospinal fluids of poliomyelitis and encephalitis show abnormal changes in practically all our cases.
2. The fluids of poliomyelitis and encephalitis present no specific characteristics.
3. Fluids from cases of encephalitis generally show a higher albumin-globulin content than do the fluids of myelitis or of the abortive cases.
4. Fehling's solution is reduced by all fluids but not in equal degree.
5. Examination of the spinal fluid is the most important factor in clearing up the diagnosis in abortive and preparalytic cases.

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[12] Du Bois and Neal: Summary of Four Years of Clinical and Bacteriologic Experience with Meningitis in New York City, Am. Jour. Dis. Child., January, 1915, ix, 1, and this book, p. 13.

[13] Frissel, L. F.: Jour. Am. Med. Assn., 1911, lvi, 661.

INFANTILE SCURVY: I—THE BLOOD, THE BLOOD-VESSELS AND THE DIET

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The following study considers infantile scurvy from a point of view which is somewhat unusual. Although it includes a chapter on diet, it is nevertheless chiefly concerned with scurvy as a hemorrhagic disease. This phase of the subject seemed worthy of investigation, as hemorrhage is the typical clinical symptom of scurvy, whether it manifests itself in bleeding into the gums, or as the equally well-recognized subperiosteal hemorrhages of the long bones. Indeed, some authors follow Barlow in considering infantile scurvy under the group of the hemorrhagic diseases, believing the hemorrhages to be the primary disturbance. There has been, however, as far as we know, no study of the blood in this disease, if we except some estimations, scattered through the literature, of the hemoglobin or of the blood-cells. We have devoted our attention especially to the question of the coagulability of the blood, with the object of ascertaining whether there is any marked change in this particular and whether a disturbance of this function can account for the various hemorrhagic symptoms. As it is now known that coagulation is closely associated with the platelets of the blood, an examination of these cells was also carried out. In addition a routine examination was made of the red and the white blood corpuscles and of the hemoglobin. In the course of this study our attention was gradually directed from the blood to the blood-vessels. We found it urgent to seek some means by which we could distinguish between hemorrhages due to a weakness of the blood-vessels and those which are the result of some defect of the blood itself.

As may be supposed, no intimate study of this kind could be pursued without having interesting clinical phenomena present themselves for consideration from time to time. We shall not, however, consider infantile scurvy from a clinical point of view, as the disease has been so frequently and thoroughly treated in all its symptomatology, but shall merely discuss a few clinical phenomena which seem to be of interest. The same is true as regards diet. This question will also not be taken up in detail, but be discussed merely in some particular aspects. Our study in the main is based on numerous cases of scurvy which have developed in the past few years in the Hebrew Infant Asylum. We are dealing, therefore, with a group of institutional infants concerning whom we have clinical records for a long period previous to the onset of the disease, and whose welfare we have been able to follow for many months following their recovery. A number of these cases developed in the course of an attempt to dispense with the giving of orange-juice. In view of the fact that pasteurized milk is now heated to a temperature of only 145 F., which is claimed by many (including the commission on milk standards) not to destroy its chemical constituents, it seemed that infants should thrive on this milk without the addition of fruit-juices to their diet.

In obtaining blood for the tests of coagulability, about 5 c. c. was obtained from the jugular vein and allowed to flow into a 1 per cent. solution of sodium oxalate in the ratio of 1 : 10. In no case were coagulation tests made from blood obtained from the prick of a finger or the lobe of the ear;

these methods cannot be relied on, as they introduce the disturbing factor of the thromboplastic substance of the tissue juices, and thus do not allow us to obtain an accurate estimate of the coagulability of the blood itself. The importance of this point may be realized when we add that the simple fact of not entering a blood-vessel at the first attempt, and in this way of introducing a minimal amount of tissue-juice into the aspirated blood, was found to invalidate our results. The oxalated blood was immediately centrifuged for fifteen minutes at high speed, and the plasma pipetted off for examination. The coagulation tests were carried out after the method suggested by Howell,[1] which is as follows:

Five drops of plasma are added to each of five small test-tubes. To these tubes increasing amounts (one to five drops) of 0.5 per cent. calcium chlorid made up in normal salt solution are added. The time is then noted when the plasma in the various tubes is firmly clotted. This time is designated as the coagulation time, and was noted in our records (Table 1) by a + + + sign. Lesser degrees of coagulation were marked +, which indicates the very faintest beginnings of coagulation, and + +, which indicates a jellylike consistency of the clot.

In addition to these tests, a test was made for antithrombin in many cases. For this purpose, the plasma was heated to 60 C. in order to destroy the prothrombin, and was then filtered, in order to get rid of the coagulated fibrinogen. One drop of this heated plasma was put into each of four tubes. To these tubes 1, 2, 3 and 4 drops, respectively, of thrombin was added. This thrombin was prepared from fibrin after Howell's method and was obtained in crystalline form. After the antithrombin and the thrombin had remained in contact for fifteen minutes, ten drops of a fibrinogen solution (made from cat plasma) were added and the time noted when coagulation occurred. In all these tests, whether for prothrombin or for antithrombin, control tests of normal plasma were carried out at the same time and under identical conditions.

Table 1 shows five tests of plasma from scurvy and three from control cases. It will be seen (Table 2) that two kinds of examinations were made; the one, which may be termed the coagulability test, includes the interaction of all the substances of the plasma, the prothrombin, the antithrombin, the calcium, the fibrinogen, etc., and the other, designated as the antithrombin test. Looking over Table 1 we find that the plasma of the scurvy cases generally clotted more slowly than that of the controls; in other words, that there is a slightly diminished clotting-power of the blood in scurvy. This property, however, is not absolutely constant, and therefore not an essential manifestation of scurvy. To what constituent of the blood is this diminished coagulability to be attributed? A glance at Table 2 at once negatives the possibility that it may be occasioned by an increase of antithrombin. The tests were uniformly convincing in this regard. May it then be the result of a lack of prothrombin or a lack of calcium or a deficiency of both these essential factors of clotting?

The question of a deficiency of calcium in the blood is an interesting one, not only because this substance is of prime importance in coagulation, but in view of the fact that some have maintained, as we shall see, that the calcium content is decreased in the tissues in scurvy, whereas others have found it to be increased. Before discussing this question, however, let us consider the significance of these coagulation tests from this point of view. The addition of sodium oxalate to plasma prevents clotting, as is well known, by

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[1] Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med., January, 1914, p. 76.

TABLE I

Results of Coagulability Tests of Plasma (Prothrombin)

Date	Name	$CaCl_2$ 0.5% (drops)	Time of Coagulation (min.)*	Remarks
4/3	M. N. (scurvy).........	1	12	Infant has hemorrhage into the gums and petechiae on body.
		2	10	
		3	9	
		4	9	
		5	9	
	A. B. (normal).........	1	7	
		2	6½	
		3	6½	
		4	6½	
		5	7	
5/4	M. H. (scurvy).........	1	15+	Infant has slight peridental hemorrhage and some petechiae.
		2	10	
		3	10	
		4	10	
		5	10	
	C. F. (normal).........	1	15+	
		2	15+	
		3	10	
		4	10	
		5	8	
	B. B. (scurvy).........	1	15+	Infant has swelling of femur.
		2	15+	
		3	12	
		4	8	
		5	8	
5/16	H. G. (scurvy).........	2	15+	A freshly made solution of calcium was used in this experiment.
		3	14	
		4	12	
		5	12	A mild case of scurvy.
		6	10	
		7	14	
	S. B. (normal).........	2	15+	
		3	10	
		4	10	
		5	10	
		6	6	
		7	10	
	H. C. (scurvy).........	2	15+	A mild case of scurvy.
		3	12	
		4	12	
		5	10	
		6	8	
		7	12	

* Five drops of oxalated plasma.

Epicrisis.—In these tests the tube which first showed clotting should be taken as the standard, as it represents the optimal recalcification. It will be noted that coagulation was but slightly delayed in the cases of scurvy.

means of precipitating the calcium in the blood. It should be understood that in the ratio in which we have added it to the plasma, it is not sufficient to precipitate all the calcium in the blood, some calcium still remains in solution. This becomes evident if we compare the results of adding the usual strength (1 per cent. of oxalate) and twice this percentage to specimens of the same blood.

TABLE 2

Results of Examinations for Antithrombin

Date	Name	Thrombin (Drops)	Time of Coagulation (Min.)
4/3	M. N. (scurvy)	1	16
		2	4
		3	3
		4	3
	B. G. (child who frequently developed ecchymotic spots)	1	14
		2	5
		3	3
		4	3
	A. B. (normal)	1	20
		2	6
		3	5
		4	5
5/4	M. H. (scurvy)	1	26
		2	6
		3	6
		4	6
	C. F. (normal)	1	20
		2	6
		3	6
		4	6
	B. B. (scurvy)	1	14
		2	4
		3	4
		4	4

Epicrisis.—Evidently the antithrombin is not increased in scurvy; further tests confirmed these results.

Table 3 reproduces an experiment of this kind. This table shows that although there is no difference as to time of clotting between the series to which 1 or 2 per cent. oxalate was added, there is marked difference between them as regards the tube in which the clot first occurred. In the tests in which 1 per cent. sodium oxalate was added, there was rapid clotting when 3, 4 and 5 drops of calcium solution were added to the plasma. On the other hand, where the plasma had been made with a 2 per cent. oxalate solution it required 5 drops of the calcium solution to recalcify the plasma sufficiently to bring about a clot; 3 or 4 drops of calcium were absolutely insufficient to bring about this result. In other words, the clotting-time remains unaltered in our tests whether we add more or less of the sodium oxalate solution; the only difference is that, if we add more oxalate, it is necessary to recalcify with a correspondingly greater number of drops of the calcium solution. There is one other physiologic fact which we must take into consideration in judging these coagulation tests, namely, that in the clotting of the plasma the amount of calcium necessary to produce a clot varies in direct ratio with the amount of prothrombin present. It is therefore not correct to consider these two factors separately—to speak, for instance, of a deficiency of calcium; for it is only a relative deficiency that has any clinical importance. If the plasma is diluted to one-half, less calcium is required to produce a clot; if it is diluted to one-fourth, still less calcium is required. We shall not enter into the details of the experiments which demonstrate this interesting phenomenon, as we expect to report on it elsewhere in another connection.

TABLE 3

COAGULATION TEST OF PLASMA PREPARED WITH A WEAK AND STRONG OXALATE SOLUTION

Date	Strength of Oxalate Added (%)	$CaCl_2$ 0.5% (Drops)	Time of Coagulation (Min.)*	Remarks
6/26	1	1	36+	Both tests were made with the same specimen of blood.
		2	36+	
		3	7	
		4	6	
		5	6	
	2	1	36+	
		2	36+	
		3	36+	
		4	36+	
		5	6	

* Five drops of oxalated plasma.

EPICRISIS.—The strength of the oxalate solution does not affect the time of clotting, but does influence the amount of calcium which it is necessary to add in order to bring about coagulation. The calcium is therefore not entirely precipitated from the blood by the 1 per cent. solution.

With these facts before us as regards both the decalcification of plasma by oxalate and the interrelation of calcium to prothrombin in the clotting process of the blood, let us return to Table 1 and consider whether it can furnish any information as to a possible deficiency of these two substances. Although it does not enable us to state whether or not there is a slight decrease of prothrombin, it does enable us to judge whether or not a *relative* deficiency of calcium exists, that is to say, relative to the prothrombin. From a physiologic standpoint, as we have stated, this is the only type of calcium deficiency that is important—a condition of negative calcium balance, in which the calcium is insufficient to bind all the prothrombin, and in which, as a consequence, coagulation is retarded. Such a condition does not exist in infantile scurvy. *The slight defect in coagulability cannot be attributed to a relative insufficiency of calcium.* Had such been the case, we should have found it necessary to add an increased amount of calcium to the plasma in scurvy cases as compared with the normal control; the clotting would have occurred in the tubes containing the larger amounts (five or six drops) of calcium solution. The tests show, however, that no increased addition of calcium was necessary. Whatever may be the finer physiologic cause, the slight defect in the clotting-power of the blood, which we have noted, plays but a secondary rôle in this disease; the characteristic hemorrhages are the result, as will be shown below, of quite a different pathologic condition.

A few simple tests of coagulation were made with unaltered blood. For this purpose ten drops of blood were dropped from the syringe into thoroughly cleaned, flat-bottomed test-tubes, 1.5 cm. in diameter, and the time of clotting ascertained. The normal coagulation time of blood tested in this manner is about six minutes. The tests of the blood of the scurvy cases varied between four and one-half and eight minutes and were therefore considered to correspond to the tests with plasma.

In addition to the coagulation-time of blood, the bleeding-time has been emphasized, especially by Duke.[2] This term refers to the time which it takes the blood to cease flowing after a puncture is made into the tissue. For this purpose a prick may be made in the lobe of the ear and the time noted when the blood ceases to flow. It is evident, according to what we have stated above, that a test of this kind includes at least two factors of coagulation, the coagulability of the blood as well as the thromboplastic substances of the tissues, and therefore that it is not identical with a test of the coagulation time of the blood or of the plasma. In two cases, one mild, the other pronounced, there was found to be a prolonged bleeding-time. The blood kept flowing from the puncture for fully five minutes and showed a deficiency in clotting. In other cases, however, in which the disease seemed equally advanced a prolongation of the bleeding-time was not met with, so that we must conclude that this abnormal condition may occur, but cannot be considered characteristic of the disease.

Table 4 shows the result of the examination of platelets. These tests were carried out by means of the staining solution recommended by Wright and Kinnicutt, the blood being obtained from the finger-tip. Although we were unable to obtain uniform counts from day to day, nevertheless the number of platelets was found in all cases to fall within normal limits. There was no marked decrease in the number of platelets such as has been described in connection with some hemorrhagic conditions and which might account for a tendency to hemorrhage. It will be noticed that in each case two counts were carried out.

In other particulars the blood shows the changes which are commonly associated with secondary anemia (Table 4). There was a relative as well as an absolute deficiency in the hemoglobin, a slight decrease in the number of red blood-cells, occasionally the appearance of a nucleated red cell, and an increase in the number of leukocytes. It is impossible to state whether this moderate leukocytosis has any specific connection with the disease, or whether it is a reaction to some toxic substance existing in the circulation and should be brought into relationship with the slight febrile reaction which has been frequently noted to occur as one of the phenomena of infantile scurvy. In connection with these blood-changes we wish to call attention to the regeneration of hemoglobin and of red cells during the convalescence of the disease. It will be noticed (Table 4) that in one instance there was an abnormal increase in the red cells (polycythemia), the count reaching over seven millions on two successive days.

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[2] Duke, W. W.: The Pathogenesis of Purpura Hemorrhagica with Especial Reference to the Part Played by Blood-Platelets, Arch. Int. Med., November, 1912, p. 445.

TABLE 4.

THE PLATELETS AND OTHER BLOOD-CELLS IN SCURVY.

Name	Date	Plate-lets	Leuko-cytes	Erythro-cytes	Hmgl. % (Sahli)	Remarks
M. H.	5/ 3	280,000	......	....	..	
	5/ 4	248,000	10,000	....	..	
	5/ 5	......	......	....	..	Boiled orange juicegiven.
	5/ 8	......	......	4,300,000	35	
	5/16	......	15,900	....	..	
	7/ 2	......	6,800	5,456,000	40	Well but pale.
A. L.	5/ 3	300,000	......	....	..	
	5/ 5	......	21,000	....	..	
	5/ 9	......	......	5,480,000	..	
	5/11	382,000	......	....	..	
	5/13	......	......	....	65	
	5/16	......	11,500	....	..	
H. C.	5/ 8	320,000	20,000	....	..	
	5/ 9	......	......	5,340,000	..	
	5/13	......	......	....	70	
	5/16	362,000	......	....	..	
B. B.	5/ 4	496,000	21,000	....	..	A severe case.
	5/13	585,000	14,000	3,200,000	70	
	5/18	......	17,600	....	..	
	7/13	......	40,000	7,672,000	82	Has gained well lately.
	7/15	......	......	7,640,000	88	
H. Y.	5/15	560,000	......	....	..	
	5/16	424,000	......	....	..	
	7/ 9	......	......	5,750,000	45	

THE BLOOD-VESSEL: CAPILLARY RESISTANCE TEST

What then is the cause of the bleeding which dominates the clinical picture of this disease? When it was found that the hemorrhages could not be accounted for by a defect in the clotting-power of the blood, we directed our attention to some disturbance of the blood-vessels. For the purpose of this investigation we devised a "capillary resistance test,"[3] which is carried out as follows:

A blood-pressure band is placed about the infant's arm in the usual manner. We made use of the Tycos apparatus for this purpose and raised the pressure rapidly until it reached the 90 mark. After the pressure had been maintained at this level for three minutes the band was removed, and when the blueness of the arm had faded an examination was made for petechial spots. The principle of this test is simple and must be evident at once. It consists merely in putting an excess of pressure on the capillaries and venules and observing whether the vessels allow the blood to escape readily. Ninety degrees pressure was selected as the optimum, because it was found that a less degree did not regularly produce venous congestion of the arm, which it is essential to bring about. In a few cases, due to an unexplained cause, there was an increase of arterial pressure reaching 100 or somewhat higher, so that raising the resistance to 90 did not produce cyanosis of the extremity; in a case of pertussis the pressure reached 125 when the infant cried. In such cases it was of course necessary to raise the pressure somewhat higher, as the entire test loses its efficacy if the return flow of the blood is not completely occluded. Numerous cases of scurvy were tested by this means and in addition, many normal cases served as control observations. It was found beyond question that the scurvy cases reacted abnormally. In these infants the forearm would show many petechiae; in some cases they were sprinkled over the entire surface whereas in normal infants they were almost always absent or there were but few to be seen.[4] In the case of an infant 18 months old, who developed a mild scurvy in spite of receiving vegetables, the test was negative; in all others it was positive, and persisted in some instances after all definite symptoms had disappeared. Where an eruption, such as eczema or prurigo, was present on the forearm, the petechiae developed especially at the site of the lesions.

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[3] Hess, A. F.: Proceed. Soc. Exper. Biol. and Med., 1914, xi, 130.

The capillary resistance test is brought forward not as a specific test for scurvy, but rather as a method of bringing to view a weakness of the vessel walls whatever may be the cause. We have found it to be positive in various cases of toxemia, and it will no doubt be found to be present wherever the vessel walls are weak; for example, in some of the purpuras which occur more frequently in adult life.

In connection with scurvy a study of petechiae is of the greatest interest. In our experience they have been present very frequently in the earliest stages of the disease. It is probable that their presence has not been emphasized in descriptions of this disease because they have not been sufficiently sought for. This year we had an exceptional opportunity of investigating this sign, as well as other early symptoms of the disease, when, as stated above, we were making observations on the group of infants fed with milk which had been subjected to a moderate degree of temperature. As may be imagined, not only were petechial spots sought for almost daily, but all other early signs of disturbance of nutrition were examined for with regularity. In adults in the course of various purpuras, superficial petechial spots are found especially in the skin of the lower extremities. This can probably be explained by the fact that these vessel-walls give way as the result of the increased pressure of gravity, brought about by the standing posture. We should naturally expect the distribution of petechiae in infants to be somewhat different on account of their horizontal posture. Such proved to be the case. Petechiae were most frequently seen on the upper part of the back and neck, and on the chest, and more often on the upper than on the lower extremities. There was, however, no part of the body that can be said to have been entirely spared. They were found in the mucous membrane of the hard and soft palate, and also in several instances on the palpebral conjunctiva, appearing just as they do so typically in cases of bacterial infection of the blood. It is probable that we must consider the blood in the urine, especially the minute hemorrhages which can be detected only by microscopic examination, merely as an evidence of petechiae into the internal organs. Post-mortem examinations show the same wide distribution of small hemorrhages. In almost all cases they have been found in the pleura, peritoneum, pericardium, in addition to the large extravasations which are found beneath the periosteum.

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[4] No importance should be attached to the petechiae which appear just below the pressure band, as these are found in most cases, normal as well as abnormal. After removing the bandage a sufficient interval should be permitted to elapse for the arm to assume its normal color so that the minute hemorrhages can be readily seen. Pressure tends to bring them out prominently. It is probable that the test can be carried out by simply applying a tourniquet for three minutes sufficiently tight to cause venous congestion but not to obliterate the radial pulse.

Besides the petechial hemorrhages, we must consider edema as being typical of an increased permeability of the vessel-wall. In scurvy we frequently find not only petechiae but also edema. Edema of the eyelids and of the ankles is well known as a clinical manifestation of the scurvy of infants as well as of adults. Post-mortem examinations also confirm the increased permeability of the vessels in this regard; fluid is commonly found in the pleural, the peritoneal, and the pericardial cavities, as well as a marked increase of fluid (noted on incision) in the subcutaneous tissues.

A comparison of scurvy and hemophilia from this point of view brings out a striking contrast. In hemophilia we do not find petechial spots scattered over the surface of the body, nor is edema a symptom of the disease. On applying the "capillary resistance test" in cases of hemophilia—we have tested three cases of this disease by this method—it is found that petechiae are not induced by this increase of intravascular pressure. In other words, in hemophilia the vessels seem to be normal. The clinical symptoms of the two diseases bring into sharp relief this distinction in the pathogenesis of the hemorrhages. In scurvy the hemorrhages are generally minute and numerous; in hemophilia the hemorrhages are few and extensive, because when the blood does escape from the vessels an excessively large amount is poured out owing to its defective coagulability. We shall not, however, enter on this subject in greater detail, as it will be considered in the future as a study of hemophilia.

When one makes a subcutaneous puncture, for example, into the abdominal wall, in the case of an infant suffering from scurvy, it was found that very often a small hemorrhage develops at the site of the puncture wound. This is not the case when one makes a hypodermic or subcutaneous puncture in a normal person. It was thought that this observation might be of value in distinguishing early cases of scurvy, and, in fact, the first tests carried out with this object in view promised well for this method. In this case (that of an infant, 6 months old, admitted to the asylum in the spring of 1913) puncture tests of this kind resulted in a hemorrhagic reaction; as soon as orange-juice was given the reaction ceased. More extensive trials with this puncture test, however, showed it to be unreliable. This is due partly to the fact that although a minute hemorrhage occurs, it does not always appear at the surface, but is concealed beneath the integument. Notwithstanding the fact that subcutaneous puncture cannot be relied on for diagnosis, it is of interest to note the difference between the reaction to this simple procedure of normal infants and of those afflicted with scurvy; it may prove to be of diagnostic value when hemorrhage results. Possibly it is merely an evidence of increased bleeding-time, or the subcutaneous tissues as well as the vessels may be involved in this disease.

Clinical Observations

In a consideration of scurvy the period which may be termed prehemorrhagic or prescorbutic is the most important. By this is meant the time when the first nutritional changes take place and before the diagnosis can be made. This interval varies in length according to individual susceptibility and according to the diet which the infant received. In our cases it varied from about four to seven weeks. This prescorbutic period, during which scurvy is latent, frequently passes unnoticed and indeed in many cases does not furnish any sign or symptom whatsoever by which it can be recognized. The question also may be raised as to just when scurvy can be considered cured, and whether there is not a postscorbutic latent period. Are we to consider an infant who has had scurvy normal, as soon as all the signs and symptoms of illness have disappeared? It is probable that although the symptoms of scurvy disappear with almost miraculous rapidity within even a few days of the administration of orange-juice or fresh vegetables, many of the cells of the body do not become normal for a far longer period. This conclusion would follow from the fact that when we apply the capillary resistance test to infants who have had scurvy, we find that they react positively, that the vessels show an abnormal permeability, for some weeks after all symptoms have disapeared. Again, a case such as the following leads us to this opinion:

J. H., April 19, 1913, showed signs of scurvy; the left femur was swollen and tender, also the tibia. There was swelling of one eyelid. These symptoms developed in spite of the fact that breast-milk had been given for eight days previously. On April 19 orange-juice was given. Although the swelling and the tenderness of the bones rapidly disappeared and the child gained in weight and appeared well, it again developed (on May 9) a hemorrhage over one eyelid.

In other words, although the child seemed to be absolutely well, there evidently was still a tendency to hemorrhage. Furthermore, the liability to recurrence or to relapse in scurvy would suggest that the tissues do not return absolutely to the normal state, although this might also be interpreted as evidence of a susceptibility on the part of the individual infant. In the literature recurrences are mentioned as occurring very exceptionally. This view is probably based on the fact that following the development of scurvy, all precautions are taken to prevent a recurrence. During the last few years we have observed two instances in which infants have twice had scurvy; indeed, it seems logical to suppose that, given favorable conditions, a second attack will occur. The following is a case in point:

Infant 6 months old, weighing 10½ pounds in September, 1913, developed swelling above and below the knee, accompanied by marked tenderness. It had been getting Schloss milk for one month. When lemon-juice was given and the food changed to two-thirds pasteurized milk and one-third barley-water with sugar, the symptoms rapidly disappeared. The lemon juice was discontinued at the beginning of January. Six weeks later tenderness of the lower extremities was noted and a hemorrhagic reaction to subcutaneous puncture. In addition to the milk, the infant had received cod-liver oil and phosphorus for three weeks. Hemorrhage of the gums developed some weeks after this. At the time of the first attack of scurvy, the infant was 6 months old and weighed 10¼ pounds; at the time of the second attack it was 11 months old and weighed 14½ pounds.

Although we do not wish to consider the symptomatology of scurvy, as it has been thoroughly portrayed so often, nevertheless we shall devote a few words to the all-important subject of the early symptoms. We have had an exceptional opportunity to observe the disease from this point of view, especially during the past year where, for a time, we were watching a group of infants to ascertain whether they would show any scorbutic signs on discontinuing the giving of fruit-juice. Writers on this subject have described various symptoms as constituting the earliest signs of the disease. Our experience in this regard has been as follows:

In many cases we found the petechial spots, referred to above, as the first evidence of incipient scurvy; the classical tenderness of the extremities was very often called to our attention by the nurse; in other instances the pallor of the infant led to the diagnosis of scurvy. This peculiar pallor has been noted by many observers and has been attributed by some to a loss of blood due to the hemorrhages. This correlation, however, does not seem to exist. The case among our series which had the greatest amount of hemorrhage, a true hematuria, nevertheless did not show marked pallor. It is probable that the loss of color is due rather to some disturbance of nutrition. Loss of appetite occurred early in some cases, but was not a constant feature. In others marked sweating was noted. English authors have laid especial emphasis on the early occurrence of red blood-cells in the urine. In view of this opinion especial attention was given to this point, to determine whether it constituted a reliable sign of the onset of this disease. It was, however, found quite inconstant. In several cases frequent examinations were made of centrifugalized specimens of the urine and no red cells whatsoever found, although at this time there was typical hemorrhage into the gums. In numerous instances we could observe no sign previous to these hemorrhages into the gums, occurring where teeth were present or were about to erupt. In some of these cases the hemorrhage into the gum was posterior to the teeth, and could be seen only when the mouth was wide open. Hemorrhages frequently can be artificially produced by sharp rubbing of the gums, a little expedient which will be found to be of diagnostic value in some cases. The distribution of the hemorrhage depends merely on chance circumstance. For example, it seems reasonable to ascribe the fact that the sub-periosteal hemorrhages involve the femur most frequently, to the frequent handling of the things in the course of the diapering of the infant. As has been stated above, almost any region may be involved in the hemorrhage. Not infrequently it may develop over the skull-bones. Finkelstein mentions that hemorrhage has been found post mortem in the dura mater. In one of our cases (L. S.) in which convulsions supervened in the course of the disease, the blood-tinged cerebrospinal fluid led us to believe that we were dealing with a case of this nature. This paragraph may be summarized by stating that there is no symptom which can be considered as pre-eminently the primary sign of scurvy; each varies in its time of appearance. *We would, however, add to the category of early signs of this disease the development of numerous petechial spots.*

According to our experience, the infectious diseases, including the prevalent infection termed " grip," do not pay an essential rôle in the production of infantile scurvy. In 1912 a very severe and extensive epidemic of this infection spread through the infants' ward at about the same time that numerous cases of scurvy developed, but a careful analysis showed that there was no relation between the two diseases. It is probable, however, that any disturbance which decreases the vitality tends in a measure to the development of scurvy. Many of our patients had rickets as well as scurvy. The question of the interrelationship of these two diseases has been discussed pro and con since scurvy was first described by Barlow. In the statistics compiled by the American Pediatric Society, 45 per cent. of the scurvy patients suffered from rickets. In both cases of recurrence of scurvy which occurred in our wards it should be noted that rickets was also present.

Although the relationship between rickets and scurvy is questionable, there is another disease or diathesis which we believe to be definitely associated with the incidence of infantile scurvy. We refer to the condition described by Czerny as " exudative diathesis," a term which implies a predisposition to develop exudations into the skin or the mucous membranes; prurigo, " milk crust," some cases of eczema, recurrent bronchitis, etc., are some of the numerous manifestations of this congenital condition. It is generally agreed that a predisposition to scurvy exists, that although a large number of infants may receive the same diet only a few develop scorbutic symptoms. This has also been our experience in the infant asylum. Closer investigation revealed the fact that *almost all the cases that developed scurvy had exudative diathesis;* this was true in ten of the twelve cases, and in the two exceptions our records are incomplete in this regard. The association was so evident, that we have no hesitation in considering that there is a close inter-relationship between the two conditions, that *exudative diathesis implies not only a diathesis for exudations, but also for the development of scurvy.* It is impossible to state whether this clinical relationship is one of summation or whether the two pathologic states are dissimilar, the exudative diathesis merely rendering the tissues more vulnerable toward scurvy. In this connection it is probably not without significance that the blood-vessels in exudative diathesis also show a decided weakness, an increased permeability, as judged by the capillary resistance test; petechiae can be produced by pressure in such cases not only during the scurvy, but to a less degree long after the scorbutic symptoms have disappeared.

In order to test the vital activity of the cells in scurvy the Schick test was carried out on six patients. This test consists in injecting intracutaneously 0.1 c. c. of a 1:10,000 solution of diphtheria toxin. In four patients ranging in age from 8 to 13 months there was no reaction; in other words, a sufficient amount of diphtheria antitoxin had been elaborated in the body to neutralize the toxin which was injected; in two cases the reaction was positive. From these tests it would seem that in spite of the marked nutritional changes, the cells are able to elaborate some of the important immune bodies. It would seem that in this disease the functions of certain cells are affected far more than those of others. For instance, the very changes so typical in the bones are brought about by the association of a lack of functioning of the osteoblasts combined with a normal activity on the part of the osteoclasts.

Diet

The infants in the group which we are particularly considering were being fed on various preparations of milk, and their diet was in no wise changed, excepting, as has been stated, that an attempt was made to do without orange-juice. Most of them were receiving mixtures of milk and barley water, the milk being "Grade A Pasteurized," that is, heated for thirty minutes to 145 F. Some were receiving malt soup, others Schloss milk and a few Eiweissmilch. It is interesting to note that all four children who were being fed on malt soup developed scurvy; this may have been mere chance, as these infants all had exudative diathesis, but can also be accounted for by the fact that in the preparation of this food the milk had been heated twice, the pasteurized milk having been brought to the boiling-point. As Neumann pointed out, this two-fold heating probably plays an important rôle in the production of scurvy. It is also possible that the malt should not be disregarded in this connection. Whether cereal, which was used in the form of barley-water, tends to the production of scurvy has never been definitely determined. This point must be borne in mind, however, in view of the classical paper of Holst and Froelich[5] on experimental scurvy produced by the giving of cereals, and on reflecting how large a part proprietary foods played in the statistics of the American Pediatric Society. As regards the cases which developed on an Eiweissmilch diet, little need be added. This is not a criterion of the efficacy or usefulness of this food, which was devised not as a permanent diet, but as a therapeutic agent.

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[5] Holst, A., and Froelich, T.: Ztschr. f. Hyg. u. Infektionskrank., 1912, lxxii, 1.

During the past three years we have had a considerable number of cases of scurvy develop in infants being fed on pasteurized milk. In 1912, when the first cases were noted, we were pasteurizing the milk in our own diet kitchen, heating it to a temperature of 165 F. for twenty minutes. At this time several cases of scurvy developed, owing to the fact that through an oversight orange-juice was not given. During the past year we have been supplied by one of the large dealers with a pasteurized milk that has been heated only to 145 F. for thirty minutes. Nevertheless, we had several cases of scurvy develop on this diet. In most of the cases the infant was receiving two-thirds milk and one-third barley-water, with the addition of sugar. In two instances, whole milk was being given without the addition of barley water. There were other patients in the same ward on the identical diet who did not develop scurvy. In other words, the pasteurized milk was not the sole factor in the production of the disease. That the pasteurization did play an important rôle, however, was shown by substituting raw, unheated milk for the pasteurized milk, the formula and the amount of food remaining unchanged. In this case the scorbutic symptoms began to disappear within a week of giving raw milk and had altogether vanished in two weeks. Whatever the predisposing factor of scurvy (this infant had exudative diathesis), a case with this clinical course can be interpreted only as being, in a large measure, the result of pasteurized milk.

Raw cow's milk must not, on the other hand, be considered as having potent antiscorbutic properties. Its effect cannot be compared to the miraculous change which is brought about by giving orange-juice. This is especially striking when we take into consideration the small amount of orange-juice necessary to bring about a cure and compare it with the large amount of raw milk which is given. Raw milk, however, contains sufficient of the essential substances to prevent the development of scurvy.

It is possible that in addition to the pasteurization there are other factors in connection with the milk which enter into the causation of scurvy. Plantegna has laid emphasis on the freshness of the milk both before and after pasteurization. This factor may be found to resolve itself into a question of reaction, of greater or less acidity, which experiments on animals have shown to be of importance. Against the justification of attributing scurvy to the heating of milk, the favorable statistics given by Variot and other French authors, including thousands of cases of infants fed on boiled milk, have always seemed conflicting evidence. Whatever may be the explanation of their results, it must be remembered that these statistics refer to patients treated in the dispensaries, and that they were not observed under ideal conditions. There can be no doubt that milk loses some of its antiscorbutic qualities as the result of heating; pasteurized to the degree which we are considering, 145 for thirty minutes, it seems to lose only a portion of its essential properties. The cases that developed in our wards on this diet were for the most part very mild. They showed petechial hemorrhages, some tenderness of the bones, a very slight degree of peridental hemorrhage, but they did not evince a tendency to progress. There seems to have been almost, but not quite, a balance between the demands of the body and the supply in the diet of the essential substances on which scurvy depends. They were not all, however, of this mild or immature type of case; two of the severest cases developed in infants receiving two-thirds pasteurized milk.

Dietetic Therapy

We have come to a consideration of dietetic treatment, which at once suggests the efficacy of orange-juice. Last year we gave, as routine, orange-juice that had been boiled for five or ten minutes and found that we were able to obtain satisfactory results. One ounce was given daily and, and as far as we could judge, boiling did not lessen its therapeutic value. This year, for orange-juice we substituted the juice of orange-peel, which was prepared as follows:

The orange-peel was finely grated and 1 ounce of it was added to 2 ounces of water, a small amount of sugar being added to overcome the slightly bitter flavor. The juice of orange-peel seems to serve the same purpose as the juice of the orange itself. It is being used at the asylum at the present time, and after a trial of several months, we have come to the conclusion that it has marked antiscorbutic power. At first we made use of the peel to test its value, but have continued its use because it allows us to serve the oranges to the older children in the institution, and in this way is somewhat economical.

According to our experience, the efficacy of vegetables cannot be compared to that of orange-juice. Two cases of scurvy developed among the infants over one year whose diet included vegetables, mainly carrots. It is impossible to state how much vegetables the nurses gave these two children; there is no reason to believe, however, that they received less than the twenty-eight other children in the ward. It is probable that they had a peculiar susceptibility to scurvy; one had exudative diathesis. When we reflect that sporadic cases of beriberi have been reported in which vegetables had been given that had been cooked for a long time, we must consider whether it is not possible that vegetables may also lose their antiscorbutic properties if cooked to a high degree. The experiments of Holst and Froelich[5] would also seem to caution us in this regard; they found that the juice of white cabbage lost its antiscorbutic value when heated even to 60 C. for ten minutes.

One of the patients, an infant receiving malt soup, developed scurvy in spite of the fact that it was given a teaspoonful of cod-liver oil three times a day for one month before the disease manifested itself. It will also be noted that in the case of recurrent scurvy which we cited, the patient had been receiving cod-liver oil for some weeks previous to the onset of the second attack. Evidently this valuable therapeutic agent cannot be relied on as an antiscorbutic. This is of interest in view of the experiments of Osborne and Mendel[6] showing the ability of cod-liver oil to promote growth in rats which had been stunted by means of a standard diet. Olive oil certainly does not possess any antiscorbutic power. In the severest case of our group the patient had obtained a teaspoonful of olive oil three times a day for a month before the development of scurvy.

The potency of potato was tried in some cases. It will be remembered that, in the scurvy of adults, the value of potato has been greatly lauded, and that epidemics of this disease have been reported to have followed a failure of the potato crop. First we made some trials with potato flour which is sold in the market; this was prepared with water and added to the milk in the same proportion as barley-water. It was soon evident, however, that potato flour cannot cure scurvy. We next employed mashed potato; a tablespoonful of boiled patota was added to a pint of water, using for this purpose the water in which the potato was boiled. In other words, instead of using a tablespoonful of barley to a pint of water, mashed potato was substituted. This was found very efficacious. The scorbutic symptoms quickly disappeared, although it did not seem to bring about the sudden change that is sometimes seen when orange-juice is given. It is probable that baked potato is just as valuable as an antiscorbutic.

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[6] Osborne, T., and Mendel, L. B.: Jour. Biol. Chem., 1914, xvii, 401.

In view of what has been outlined, at what age should we begin to give infants an antiscorbutic? There is no doubt that if an infant is fed solely on heated cow's milk the tissues begin to lose antiscorbutic substances—there is a negative balance of this material—from the very first days of life. Such being the case, it would seem that these essential substances should be supplied to the infant as soon as it is possible. As far as is known, there is no physiologic reason why orange-juice or potato should not be given in small quantities to an infant a few weeks of age. Two years ago, it was clearly shown[7] by means of an examination of the duodenal contents, that starch-splitting ferments are present in the intestine of infants at birth, and are secreted in large measure after the first few weeks of life. It would also seem worthy of trial to substitute potato water for barley-water in the mixtures of pasteurized milk which are being distributed with such great benefit by the various diet kitchens in the larger cities. This will obviate the necessity of constantly admonishing the mothers not to omit orange-juice from the daily diet of their infants.

COMMENT

The chemical processes involved in scurvy are as yet unknown. There have been a few metabolism experiments connected with this disease, but they do not agree in their results. Lust and Klocman[8] found a positive balance of mineral salts in the course of scurvy and a negative balance during convalescence. They describe a disturbance of elimination of the salts of the body, which is quite the opposite to what is found in rickets.

A more recent study of metabolism is that of Bahrdt and Edelstein[9] which is based, not on examinations during life, but on chemical analysis of the organs, after death. These authors found a decrease of ash in the bones, especially of calcium and phosphorus very much as in rickets. A test of this nature, of the organs of the body, ought to afford reliable information. It should be noted, however, that in the case in question the patient had been under treatment for six weeks prior to death and that the tissues may therefore not have been in the active stage of the disease at the time of death. Further tests must decide which of these views is correct; from a clinical point of viw, it is difficult to associate a marked deficiency of calcium salts with a disease in which fracture of the bones is a classical symptom, followed by a normal formation of callus. It should be emphasized that metabolic studies of this kind at present are necessarily incomplete, that after the various salts and organic substances are quantitatively analyzed, there is no doubt that more substances have been omitted than included in the chemical tests. This disease, furthermore, sharply emphasizes the fact that although an estimation of the caloric value of food is important, it may omit the very substances which are essential to health and life. In some of our cases the caloric value of the food was as high as 120 calories per kilo, body weight, but nevertheless there was a development of scurvy, accompanied by loss of weight and failure of nutrition.

In this connection we must mention the very interesting and suggestive studies of Funk.[10] This author has coined the word " vitamines " for substances which are essential to the health and life of the body, and the lack of which produces a group of diseases which he has termed the "avitaminosen," including beriberi, scurvy, pellagra and rickets. The vitamines, Funk asserts, are crystallized nitrogen containing bodies of very complicated structure which are chemically defined, but concerning the exact structure of which we as yet know little. They are essential to life, although present in very small amounts. Such is the definition which Funk gives of the substances which he considers play an important and even vital part in nutrition.

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[7] Hess, A. F.: The Pancreatic Ferments in Infants, Am. Jour. Dis. Child., October, 1912, p. 205.
[8] Lust, F., and Kloeman, L.: Jahr. f. Kinderh., 1912, lvxxv, 663.
[9] Bahrdt, H., and Edelstein, F.: Ztschr. f. Kinderh., 1913, ix, 415.
[10] Funk, C : Die Vitamine, 1914, J. F. B.

Similar studies have been made by others, notably Stepp,[11] who terms these substances "lipoids," by which he means substances soluble in alcohol and in ether. He also found that animals could not live when deprived of these substances. Although these "vitamines" have not been satisfactorily isolated from a chemical point of view, and exception has therefore been taken to the term, there is no doubt from experiments on animals that these substances play an important rôle in the nutrition of the body. When they are removed from the diet the animals develop various nutritional disturbances, and regain their normal condition only when they are again added to the diet. These vitamines are thermolabile and are supposed to constitute a group of which there are various members. It is probable that one of this group is the vitamine which prevents the development of scurvy. It would also seem that this material is supplied in the mother's milk, and that this accounts for the fact that nursing infants do not develop scurvy.

SUMMARY

Infantile scurvy is a disorder characterized clinically by hemorrhage, for example, the classical bleeding into the gums and the subperiosteal hemorrhages of the long bones. A study of the cause of this bleeding, which must include a consideration of the clotting-power of the blood, forms the nucleus of this investigation.

For the coagulation tests blood was aspirated directly from the blood-vessels and oxalated. This plasma showed a slight diminution in clotting-power. This defect did not seem, however, to be the result of an insufficiency of calcium. The antithrombin was not increased.

Small amounts of blood were also obtained by puncture of the finger. Examinations of this blood revealed a normal number of blood platelets. In other respects the picture was that of a simple secondary anemia, except that the hemoglobin was diminished out of proportion to the red blood-cells. A marked regeneration of these cells during convalescence, leading to a polycythemia, was also noticed.

These various departures from the norm are insufficient to account for the hemorrhages associated with the disease. The integrity of the blood-vessels was therefore investigated by means of a device which may be termed the "*capillary resistance test.*" This test consists in subjecting the capillaries and vessels of the arm to increased intravascular pressure, by means of an ordinary blood-pressure band, and of observing whether this strain results in the escape of blood through the vessels—the appearance of petechial hemorrhages into the skin. The vessels of normal infants were found to withstand, without apparent disturbance, 90 degrees of pressure for three minutes, whereas the vessels of infants suffering from scurvy gave way under this pressure. The test is not specific for scurvy, but is a method of demonstrating a weakness of the vessel walls, whatsoever may be its cause.

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[11] Stepp. W.: Deutsch. med. Wchnschr., 1914, No. 18, p. 892.

In the course of an exceptional opportunity to observe scurvy in its incipiency, numerous petechial hemorrhages of the skin or mucous membranes were frequently noted as one of the earliest signs of the disease; no sign, however, should be regarded as pre-eminently the primary symptoms of scurvy.

It is generally recognized that scurvy has not only an exiciting cause, but a predisposing cause. *The well known "exudative diathesis" of Czerny was found definitely to predispose to the development of scurvy.* Whether there are other predisposing factors remains to be determined.

Several cases of scurvy developed in infants who were being fed on milk which was pasteurized to 145 F. for thirty minutes. They were cured by receiving fruit-juices or raw milk.

Orange-juice was found not to lose its efficacy as the result of being boiled for ten minutes. The juice of the peel was successfully substituted as an antiscorbutic for the juice of the orange.

Potato proved to be an excellent antiscorbutic. It is suggested that it be added to pasteurized milk as potato-water instead of the barley-water which is now commonly used as a diluent. In this way the necessity will be obviated of giving orange-juice.

Cod-liver oil or olive oil, although given for weeks, did not prevent the development of scurvy.

INFANTILE SCURVY: II—A NEW ASPECT OF THE SYMPTOMATOLOGY, PATHOLOGY AND DIET

Alfred F. Hess

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Infantile scurvy is commonly regarded as a disorder characterized by malnutrition, by typical changes in the structure of the bones, and by hemorrhages in various parts of the body, occurring most frequently in the gums and beneath the periosteum. The descriptions of the pathologic anatomy are remarkable for their singleness of point of view, whether we consult the textbooks on the diseases of children, or special monographs on this subject. In one and all, attention is directed almost solely to the bony lesions, more especially to the alterations in the microscopic appearance of the long bones, which are constant and characteristic of the disease. On further investigation it is found that this circumscribed point of view of the pathology of this disease has been maintained almost constantly since Barlow first placed its pathology on a firm basis over thirty years ago. Barlow[1] also reported an infiltration of the muscles with serum, and "a quantity of blood-stained serum in the pleura" in some of the cases which formed the basis of his first communication. The thoracic and abdominal organs have consistently been regarded as in no wise involved, and, indeed, whether in older or in more recent descriptions, are almost invariably passed over with scant or no consideration.

As was to be expected, the pathologic anatomy of infantile scurvy has largely dominated the outlines of its symptomatology. In the clinical descriptions we read time and again of anemia and of hemorrhages, the latter appearing most frequently in the gums or beneath the periosteum, or perhaps as petechiae into the skin, or as hematuria. There is naturally some variation from this simple clinical picture; for example, slight edema of the soft parts, and the occasional fracture of the long bones. Such are, however, in general, the objective signs of infantile scurvy, and if we add pain and tenderness, and the general symptoms of malaise, we have a fairly comprehensive picture of the disorder. More recently, the Roentgen ray has added another sign to this category, and once more we find that it is in respect to a lesion of the bones. I refer to the characteristic "white line" of Fraenkel, to be seen frequently at the epiphyseal ends of the long bones in the early stages of this disorder. It is interesting to note that in Fraenkel's monograph,[2] which is devoted to a study of the changes noted by means of the Roentgen ray, there is no mention whatsoever of an examination of the heart or the lungs, and no consideration of their possible involvement in the disease.

In a previous paper[3] on this subject, the alteration in the blood and the blood vessels in infantile scurvy was considered. It was found that the hemorrhages are attributable to a disturbance of the vessel walls rather than to a defect of the factors concerned in the normal coagulation of the blood. The group of infants which formed the basis of the first report had been in the Hebrew Infant Asylum under close observation for many months, and had been fed the usual milk mixtures. The milk given was Grade A pasteurized, which is heated to 145 F. for thirty minutes. The object of the investigation was to ascertain whether milk treated in this way undergoes any essential change, and whether infants could be made to thrive on it even when orange juice was discontinued. It may be remembered that the Commission on Milk Standards, in addition to endorsing pasteurized milk from a bacteriological standpoint—a position which has justly received general acceptance—states that heating milk to this degree does not impair its nutritive properties. Our experience led us to conclude, on the contrary, that unless some antiscorbutic substance, such as orange juice, was given, a more or less mild form of scurvy developed, which yielded quickly to simple dietetic treatment—changing to raw milk. or once more adding orange juice or the juice of orange peel to the diet. This conclusion has been questioned by some, so that, in view of the importance of the subject and its wide applicability, it seemed highly desirable to pursue the study further and to determine whether signs of scurvy again made their appearance.

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[1] Barlow, T.: Medico-Chirurg. Tr., 1883, lxvi, 159.

[2] Fraenkel, E.: Fortschr. a. d. Geb. d. Röntgenstrahlen, Supplement 18, 1908.

[3] Hess, A. F., and Fish, Mildred: Infantile Scurvy: The Blood, the Blood Vessels and the Diet, Am. Jour. Dis. Child., December, 1914, p. 385.

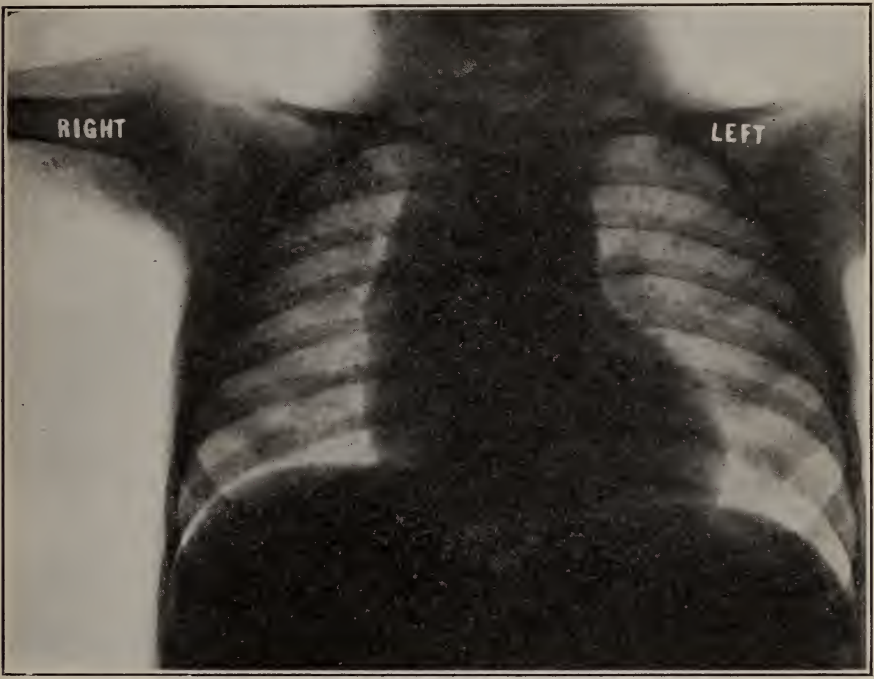

Fig. 1.—Infant (14 months old); mild scurvy. Heart enlarged greatly to the right.

Our results, from this point of view, may be summarized by the statement that, in spite of the fact that the milk was of excellent quality, that the infants received food of high caloric value, and that in many instances cereal was also given, mild scorbutic symptoms developed in almost all cases within from two to three months. The purpose of this second paper, however, is not to report these cases in detail, but to present new data noted in the course of this study, which would show not only that infantile scurvy must be regarded as a disorder involving the bones and the blood vessels, but also that there is a considerable variety of other clinical manifestations: furthermore, that this broad symptomatology affords a basis for linking it in its symptomatology and pathology to other "deficiency diseases," for example, beriberi, and finally that this relationship is strengthened by the results obtained with certain dietetic therapy.

In addition to the classical signs of infantile scurvy—the hemorrhages into the gums or beneath the periosteum—a careful examination frequently will reveal involvement of the circulatory system. Most noteworthy of these is *an enlargement of the heart, and more especially the right heart.* This sign can be elicited, when it is fairly marked, by percussion; in several instances we have detected it in this way. In three cases, the signs have been substantiated by means of the Roentgen ray. Roentgenograms of two of these taken by Dr. Charles Gottlieb are reproduced in the accompanying illustrations. The one infant (Fig. 1) was 14 months of age and had been under observation in the institution since he was about 2 months old. He had been on a diet of milk and of cereal, without the addition of orange juice, for about four months. Mild scorbutic symptoms had gradually made their appearance: pallor, stationary weight, peridental hemorrhage, and a few petechiae of the skin and of the conjunctivae. In the course of careful physical examination we were surprised to note the marked increase of cardiac dulness, which extended fully 1½ fingers to the right of the right border of the sternum, and to the left somewhat outside the nipple. The heart sounds were muffled at the apex but clear at the base. The pulse was rapid and regular. Numerous roentgenograms were taken, and all showed approximately the same cardiac enlargement. A fluoroscopic examination was made in order to see whether there was fluid in the pericardial sac, a condition which was once noted postmortem. However, there was no appreciable amount of fluid observed within the pericardium. Figure 2 shows a very similar heart condition.

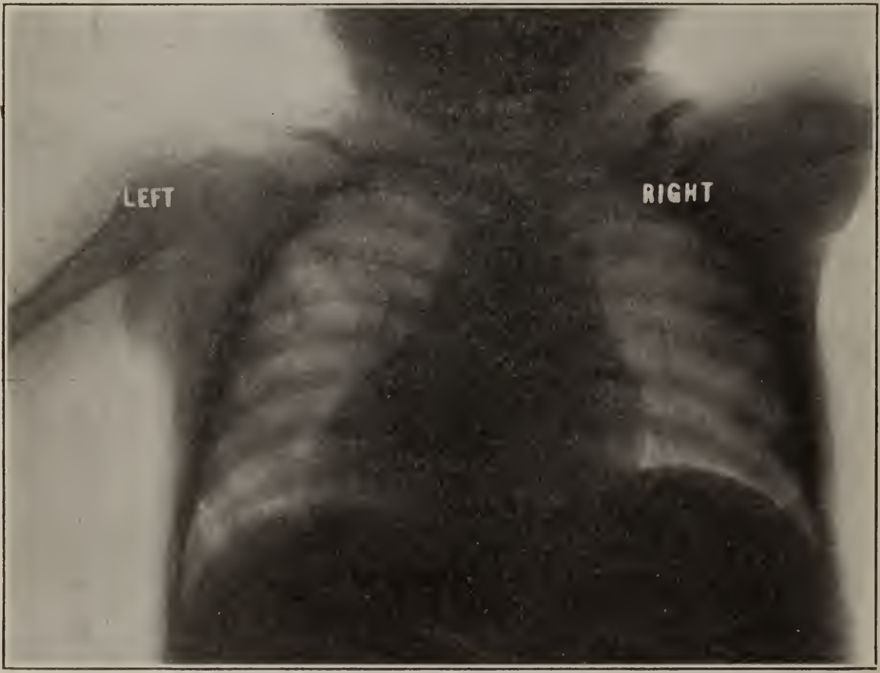

Fig. 2.—Infant (14 months old); mild scurvy. Heart enlarged greatly to the right.

Definite cardiac enlargement to the right was demonstrated both by clinical and roentgenographic examination in all three infants. In the majority of cases, however, both percussion and the Roentgen ray showed normal heart outlines. In some of these, the presence of tachycardia would seem to indicate that the circulatory system was not entirely spared. This rapidity of heart action was constant, and in the case of an infant 8 months old was found, on repeated examination, to reach 180 beats or more. In conjunction with this increased frequency of the pulse, an increased respiratory rate was noted.

As I have stated, clinical descriptions omit any mention of involvement of the heart. Barlow states that " there is nothing to note regarding the heart and lungs." Necropsy protocols usually are incomplete and unsatisfactory in this regard; there are some, however, which are significant. In the monograph on this subject by Schoedel and Nauwerck,[4] five careful necropsy protocols are reported. In two instances the heart was apparently normal. In the others we find the following record, which is passed over without comment:

1. Pericardial fluid somewhat increased, both ventricles moderately dilated, the right somewhat hypertrophic.
2. The heart showed a hypertrophy of the right and left ventricles, as well as dilation of the right ventricle.
3. The right ventricle is dilated and slightly hypertrophied, the muscles pale and tough.

In this connection the necropsy reports on animals suffering from artificially induced scurvy afford little information. The excellent works of Holst and Froelich,[5] on scurvy in guinea-pigs, and that of Hart and Lessing[6] on scurvy in monkeys, do not take any cognizance of the heart. However, the necropsies on infantile scurvy which I have cited suffice to substantiate from a pathologic-anatomic point of view the enlargement of the heart which we determined by means of clinical methods.

As is well known, edema constitutes a not infrequent symptom of infantile scurvy. This manifestation, however, has not been given the prominence which, I believe, it should be accorded. In our experience subcutaneous edema is not only one of the most constant phenomena in the symptomatology and pathology of this disorder, but deserves special consideration because it appears in a peculiar clinical form and frequently is one of the earliest indications of threatening scurvy. As can be well understood from the nature of our investigation, we had a peculiarly favorable opportunity to observe the earliest signs of this disorder. In addition to the somewhat characteristic pallor which has been so often depicted as a warning signal, we noted edema, involving most regularly the eyelids, especially the upper lids. The puffiness was met before any symptoms of hemorrhage could be found, whether bleeding into the gums or red cells in the urine, and persisted when the disorder was disappearing under appropriate dietetic treatment. The edema, however, is far more widespread. Postmortem examinations have clearly demonstrated this fact. Any one who has incised the subcutaneous tissues to reach the long bones must have noted the moist and infiltrated condition of these parts. The reason this pathologic state has played so small a rôle in the symptomatology of infantile scurvy is that the edema differs clinically from that usually encountered; in other words, it does not pit on pressure. This peculiarity is ascribable to the fact that it infiltrates the layers of the integument and the underlying muscle, rather than being diffused in the loose subcutaneous areolar tissue. It is a firm edema causing some glossiness of the overlying skin, which is rendered difficult to wrinkle or to pinch between the fingers. Indeed, at times one is in doubt whether edema is actually present, until one compares the texture of the tissues with those of a healthy infant of about the same age and weight. This firm edema occurs most frequently over the anterior aspect of the lower end of the leg. The edema, as suggested in the previous paper, is probably due to a nutritional disturbance of the smaller vessels which allows fluid to permeate the walls.

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[4] Schoedel, J., and Nauwerck, C.: Untersuchungen über die Moeller-Barlow'sche Krankheit, Jena, 1900.

[5] Holst, A., and Froelich, T.: Ztschr. f. Hyg. u. Infektionskrankh., 1912, lxxii, 1.

[6] Hart, C., and Lessing, O.: Der Skorbut der kleinen Kinder, Stuttgart, 1913.

As has been mentioned above, this transudation involves the muscles of the body as well as the subcutaneous tissue. Barlow described an "infiltration of the intercostal muscles." It is probable that when edematous fluid is poured out into the muscles of the thigh, the swelling is not infrequently wrongly diagnosed as due to subperiosteal hemorrhage. This has been our experience. I reproduce (Fig. 3) an instance in which this error occurred. It will be noted that in spite of the manifestly increased diameter of the affected thigh, there is no augmentation in the width of the bone and no typical elevation of the periosteum as is to be seen when hemorrhage has occurred, but that the difference is due solely to an infiltration of the muscular layer.

The consideration of subperiosteal hemorrhage which, as is well known, is accompanied by exquisite pain and tenderness, raised the question as to whether we are correct in ascribing all pain in the extremities to such involvement. It is difficult to answer, as the interpretation of pain in an infant is an unsatisfactory clinical task. It seemed to us after careful study, however, as if in some instances there was a tenderness of the skin or of the muscles, quite dissociated from that of the bones, which might be attributable to an involvement of the nerves. Further investigation was suggestive, although not conclusive, of involvement of the nervous system. The knee reflexes in almost all instances were markedly increased; in one case they were almost absent on repeated examination. Tests of the reaction of the nerves (median) to galvanism were not definite. An examination of the retinas, for which I wish to thank Dr. Julius Wolff and Dr. Barnert, showed edema around the disks in the two cases which were furthest advanced. As far as I can ascertain, no ophthalmoscopic examinations have been made in infantile scurvy, but it will be interesting to learn what retinal examinations in other cases will show. In this connection I may add that it is quite possible that the pathogenesis of the cardiac enlargement is an involvement of the vagus. It is difficult to understand the predisposition of the right heart to enlarge unless we associate this with a disturbance of the circulation in the lungs. The frequent occurrence of pneumonia in the course of scurvy adds weight to this view.

The syndrome which I have sketched brings infantile scurvy into actual relationship with some of the other "deficiency diseases," and more particularly with beriberi. These two diseases are commonly grouped together, merely because they have one factor in common—they can be cured by the addition of a definite food to the diet. As beriberi frequently attacks infants, it is possible to go further and to compare these two diseases in patients of this tender age. We are indebted to Andrews[7] for a comprehensive clinical and pathological account of the beriberi which is commonly encountered in the Philippines among infants who are nursed by mothers suffering from this ailment. It differs but little from the beriberi in adults described so completely by Vedder.[8] Hemorrhages occur, but are by no means constant. Cardiac dullness is increased, especially to the right, and the heart sounds are muffled. The pulse is rapid, from 130 to 170 or more, and dyspnea is almost always present. At necropsy, hypertrophy of the heart is found; "the wall of the right ventricle equals or exceeds that of the left." Edema is common, the skin having "a tough, leathery feel," and postmortem examination shows that "the subcutaneous fat is present apparently in good amount, grayish white, and very moist;" in many cases a general edematous condition is present. Examination by means of the electric current was unsatisfactory; however, a degeneration of the nerves (vagus, phrenic, intercostal, anterior tibial) was established, although this was "not as extensive as is found in cases of adult beriberi."

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[7] Andrews, V. L.: Philippine Jour. Sc., April, 1912, p. 67.

We can hardly review this summary of "infantile beriberi" without being struck by the similarity between this disorder and infantile scurvy as I have sketched it. There are some factors which are evidently dissimilar, but the differences are not such as to obscure the evident clinical and pathologic relationship between them. They show, nevertheless, that we are not dealing with one and the same disease, for it is clear that the characteristic symptom of infantile scurvy is hemorrhage, whereas that of beriberi is nerve degeneration. It is also evident that the edema is much more marked in the latter disorder. It must be borne in mind, however, that in adult beriberi there is a dry as well as a wet form, and also that exceptionally, infantile scurvy leads to the appearance of general edema. I myself have seen an instance of this kind, and others have been reported from time to time. One of the very first cases described in this country (Northrup[9]) showed edema not only of the feet and legs, but also of the scrotum. It should be investigated whether this peculiarity is to be ascribed to the idiosyncrasy of the patient or to the constituents of the diet.

Thus we see that the relationship between infantile scurvy and beriberi, which has rested on the basis of analogy, has also the foundation of symptomatology and pathology. Numerous interesting observations would seem to have rendered probable such intimate kinship. Holst and Froelich[5] noted that guinea-pigs fed on decorticated or highly milled rice developed scurvy and not beriberi; and these authors, as well as Ingier,[10] demonstrated a degeneration of the nerves in some of the animals. In man, Darling [11] reports that "in some African negroes a diet that causes scurvy in one set of men causes neuritis in others." He also showed that in adult scurvy there may be found "right sided hypertrophy and degenerative changes in the vagus and all its branches."[12] The data which I have reported serve to link some of these observations together.

It is evident that infantile scurvy, adult scurvy and beriberi have many factors in common, viewed from the symptomatic as well as from a pathologic standpoint. A test which I was able to carry out led to the conclusion that the scope of comparison may be enlarged to include the dietetic or therapeutic point of view. Although this aspect of the subject will be reported in detail in a consideration of the relation of infantile scurvy to growth, a few words concerning our results seem pertinent and of interest in this connection. It will be remembered that almost all the infants who developed scurvy were receiving cereal (farina) as well as milk. This farina is the starchy kernel of the wheat, from which the outer layers have been removed. It has been repeatedly shown that beriberi is brought about by a diet of polished rice which has been deprived of its outer layer or pericarp. It has likewise been established that the addition of rice polishings to the food suffices to effect a rapid cure of beriberi in man, or of its counterpart, polyneuritis in chickens. In view of these facts and the clinical similarity between infantile scurvy and beriberi, we decided to substitute in place of the farina a cereal containing the outer layer of the wheat. To this end we obtained middlings, the middle layer of the wheat grain, which may be compared to the pericarp of the rice. A cereal was prepared consisting of two parts of middlings and one part of flour, and was given in place of the farina and in the same amounts. The caloric value of this food is the same as that of farina, and it was taken quite as readily by most of the babies. No other change whatsoever was made in the diet. In some instances, improvement was immediate and striking; there was a gain in weight for the first time in many weeks, a brightening in the appearance and in the disposition of the infants, and a recession of the typical scorbutic symptoms. This improvement was maintained for some weeks. The effect, however, could not be compared to the miraculous change brought about when orange juice or the juice of orange peel was given. The addition of middlings, which may be designated wheat pericarp, was not able to restore the infants to their natural vigor, even when it was given daily for a month or more. In some instances, especially when only a small amount of middlings was taken, no effect was observed. Judging from these experiences, it would seem as if this layer of the wheat grain possesses a small amount of the essential antiscorbutic substance, the vitamins of Funk,[13] but an insufficient quantity to reestablish an adequate positive balance of the material. That the beneficial effect is certainly not attributable to its high fat content is evident when we reflect that the addition of cod liver oil to the diet failed to bring about the least improvement in our previous study. The future must show whether infants fed on a cereal of this character develop scurvy, even though orange juice is not added to the diet.

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[8] Vedder, E. B.: Beriberi, New York, 1913.

[9] Northrup. W. P.: Arch. Pediat., January, 1892, p. 1.

[10] Ingier, A.: Frankfurt. Ztschr. f. Path , 1913, xiv, 1.

[11] Darling, S. T.: The Pathologic Affinities of Beriberi and Scurvy, The Journal A. M. A., Oct. 10, 1914, p. 1290.

[12] In these cases the knee reflexes were increased as in our cases of infantile scurvy. These reflexes were likewise exaggerated in a group of about twenty-five men suffering from beriberi, whom I have examined recently at the government quarantine station, with the kind permission of Dr. Teague.

Conclusions

The pathology of infantile scurvy, including Barlow's classical account, is almost entirely devoted to a description of the lesion of the bones; little or no mention is made of the heart, the lungs and other internal organs. On the clinical side, attention has been focused on the evidence of hemorrhage—in the gums, beneath the periosteum, in the urine, etc.. In the foregoing paper I have approached the subject from a different point of view and attempted to show that both the pathology and the symptomatology have a broader scope and that the heart is not infrequently involved. In several instances, definite cardiac enlargement was ascertained by means of physical signs and Roentgen examination. Necropsies also show that the heart in some cases is hypertrophied and dilated. The examinations made during life, as well as those carried out after death, demonstrated that this enlargement was mainly of the right ventricle.

Another symptom which should be accorded greater significance is edema, which is almost always present to some degree. This occurs early in the course of the disease and involves most frequently the eyelids. It is often manifested as a firm edema over the lower end of the tibia, infiltrating the skin, and is readily overlooked, as it does not pit on pressure. This extravasation, which is due to nutritional disturbance of the smaller vessels, may also infiltrate the muscles. When this occurs in the thigh and causes swelling, it may be mistaken for the typical subperiosteal hemorrhage.

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[13] Funk, C.: Die Vitamine, Wiesbaden, 1914.

It would also seem probable that the nerves are involved in infantile scurvy. The knee jerks are increased, and there are indications of superficial tenderness of the limbs and neuro-edema of the optic disks.

In the light of this symptomatology and pathology, infantile scurvy assumes actual relationship to beriberi, in which the right heart is also hypertrophied and the nerves invariably involved. This correlation was emphasized still further by a dietetic test modeled after the treatment of beriberi, which, as is well known, is brought about by a diet of polished rice which has been deprived of its pericarp, and is cured by the addition of rice polishings to the diet. In our study, the addition of middlings—the pericarp of the wheat—caused in many instances a prompt amelioration of the symptoms, although it did not suffice to cure the disorder. This indicates the advantage of giving a cereal which contains the outer layers of the grain when the diet includes no other antiscorbutic food.

THE PATHOGENESIS OF CASEIN CURDS IN THE STOOLS OF INFANTS*

Alfred F. Hess

(*Reprinted from the American Journal of Diseases of Children, June,* 1913. *Vol.* 5, *pp.* 457-465)

The subject of this paper is one that has interested pediatrists both in this country and in Germany for many years, but was brought forth anew for discussion a few years ago. It is hardly necessary to review the history of investigations in this field. The articles have been many, and almost all have included a summary of the numerous investigations which have considered the problem of casein digestion. It is well known that although Biedert regarded protein indigestion as the main obstacle to successful artificial feeding of infants, this conception has been completely disavowed by his fellow countrymen. The question was not allowed, however, to slumber long, but was aroused again by Talbot,[1] who described large, hard masses in the stool, which he termed casein curds, believing them to be composed mainly of the casein of the milk. Without enumerating the various writers who immediately took sides on this important question, it should be said that Meyer and Leopold,[2] Southworth and Schloss,[3] Ibrahim,[4] Brennemann,[5] and Courtney,[6] published investigations for or against this partial retrogression to the old Biedert idea. As the result of intensive work on this subject, advance was rapidly made. Although no attempt will be made to detail the various experimental studies in this field, some few must be mentioned. In the first place, it was definitely shown by Talbot by means of various biologic tests that these curds do contain casein. It was, however, impossible by means of sensitive precipitin and hemolytic reactions to estimate how great a part of their substance the casein constituted. Another advance, shedding light on this subject, was the almost simultaneous reports of Ibrahim and of Brennemann showing that boiling the milk prevents the appearance of these masses in the stool. These studies, however, have not entirely elucidated the question. It is argued that these bean-like masses are only in part, perhaps in small part, composed of casein, and that although protein in nature, the main protein substance consists of intestinal secretion, which is mingled with the fat of the milk. Furthermore, they do not show whether the curds are formed in the stomach or in the intestine, or what leads to their formation, or how it is that in spite of the proteolytic enzymes, they pass through the entire intestinal tract undigested.

Place of Formation of Curds

In the course of the care of a large number of infants in an asylum, this question was brought to notice, and has been a source of interest and consideration for the past year or more. It seemed almost certain that these masses in the stool are similar to those which are found in the stomach contents or in the vomitus of infants, but the difficulty lay, as is often the case, in finding a means of proving a palpable fact. In this connection, we wish to emphasize a point which we feel has not been granted sufficient importance, one which all investigators have noted, namely, that the interior of these masses is always white, although the exterior is stained brown or green by the stool. This fact would seem to afford very strong proof that these masses are formed in the stomach. For if they were formed in the small intestine beyond the point where the bile enters the duodenum, they undoubtedly would be mixed with bile, and their interior would be bile-stained. From this viewpoint, the bile may be regarded merely as a colored fluid which is injected into the upper duodenum, just beyond the pylorus. Nature provides in this way a most simple and convincing experiment, one which might well be devised were we to resort to animal experimentation for our purposes. Unformed substances which come from the stomach will certainly be mixed and tinged with the bile, whereas formed substances may remain unmixed and uncolored. If this be granted, it does not seem reasonable to dodge the conclusion that these masses must be formed before they enter the upper duodenum.

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* Read before the New England Pediatric Society, March 1, 1913.

[1] Talbot, F.: Arch. Pediat., December, 1909; June, 1910.

[2] Meyer, L. F. and Leopold, J.: Arch. Pediat., October, 1909; February, 1910.

[3] Southworth, T. and Schloss, O.: Arch. Pediat., April, 1909.

[4] Ibrahim, J.: Monatschr. f. Kinderh., 1911, x, 55.

[5] Brennemann, J.: Am. Jour. Dis. Child., May, 1911.

[6] Courtney, A. M.: Am. Jour. Dis. Child., January, 1912.

Another fact closely allied to the former and pointing in the same direction, is that the main fatty constituent of these masses is not fatty acid, but neutral fat; that the curd has not been acted on by lipase which enters at about the same point as the bile. Neither the bile nor the pancreatic fluid, therefore, has access to the interior of these masses.

A new method of approach to the question of the gastric or intestinal formation of these stool curds was afforded by the employment of the duodenal catheter. Last spring there was an infant, 3 months of age, in my hospital ward, who quite regularly passed casein curds. Its food consisted of half milk and half barley water with the addition of 5 per cent. cane-sugar; of this mixture, 6 ounces were given every four hours. For the purpose of preliminary experiment, this infant was fed solely on barley water for forty-eight hours, during which time the stools became brown and scanty, and the casein masses disappeared. It was then given by means of the duodenal catheter the same milk mixture which it had received before, and was fed very slowly five times in twenty-four hours. Duodenal alimentation was thus continued for forty-eight hours and the stools became yellow and larger, but contained no bean-shaped masses. Ordinary bottle feeding was again instituted, for the first two days merely barley water, and then the same milk mixture as originally given. Forty-eight hours after the milk was given by mouth, the casein masses reappeared in the stool. This would seem to show that these curds are formed in the stomach and disappear if the food is made to circumvent the stomach. The bile coloring test mentioned above, as well as the duodenal feeding test seem conclusive on this point.

Inhibiting Curd Formation

A series of experiments were undertaken with the view of inhibiting the formation of the casein masses. Many of these methods had been resorted to previously by others. For instance, boiling the milk (in these tests for five minutes) caused the masses to disappear. We were also able to confirm the results of others who found that 2 grains of sodium bicarbonate or of sodium citrate in an ounce of milk did away with their formation. Calcium chlorid, in 10 per cent. solution, added in the ratio of 1 to 20 to the milk was most effective, although not invariably so, in doing away with the formation of the curd masses. It was, however, not so easy to produce the curd at will; the most successful method was to give rennin at the time of each feeding. In many cases, but by no means in all, this was followed by the appearance of the large curds in the stool. Diluting whole milk with one-third barley water does not prevent or materially decrease the formation of the curds. We have had very many babies on this formula who passed casein curds, indeed, the most marked instance of curd masses which we have met with, occurred in the case of an infant who was receiving a mixture of this kind.

The question of the effect of heating the milk was entered on in somewhat greater detail. Milk was heated to varying temperature for successive days and it was found that when the milk was brought to about 170 F. the curds almost always disappeared. They then occurred only occasionally and the appearance of the stools in this regard was quite different from that when milk was given that had been heated to a less high degree. We were especially anxious to determine the effect of pasteurization at 147 F. for forty-five minutes, because this is the type of pasteurization which is being employed by various dairies, and fully complies with the latest ordinance of The City of New York. Last year for this purpose some milk was pasteurized to this degree in our own apparatus, and this year milk was purchased which was thus pasteurized by the holding process at the creamery. It was found that pasteurization at this temperature did not do away to an appreciable extent with the formation of the curds in the stools; indeed, when recently in the course of experiments bearing on this question, we put fifteen infants on raw milk, we did not notice any increase in the formation of casein curds. In this connection, it may be added that an experiment was made to see the effect of freezing the milk. It was found that the curds appeared just the same, even after milk was given which had been frozen solid for a couple of hours. The substance, therefore, which goes to form these curds acts chemically like casein, which is changed in its power of coagulability by a temperature of about 170 F.

Study of Stomach Curds

Having assured ourselves that these curds were formed in the stomach, we directed our attention to the gastric digestion. With this end in view we used the stomach tube and evacuated the stomachs of many children, some of whom frequently passed curds, and others who were normal in this respect. However, we met with the same unsatisfactory experience as Tobler,[7] who found that he was unable by this means completely to empty the stomach, and proved by means of the radiograph that some milk always remains behind. In our endeavors to overcome this difficulty, we used a large stomach tube; we irritated the pharynx with the tube or with the finger; we gave syrup of ipecac, and at times followed this by warm water given by the tube, but were unable by any of these means to incite vomiting at will. This was quite surprising in view of the marked tendency of infants to vomit at the least digestive or febrile disturbance. Indeed, we found that even when the infant vomits, the stomach does not always completely empty itself, as was shown by the fact that a second or even third attack of vomiting resulted in the renewed evacuation of large curds. So that at present it is impossible to carry out a satisfactory study of the gastric curds of infants. However, we examined the curds which we obtained by means of introducing the tube, as well as those from time to time vomited spontaneously. On comparing the size and the firmness of the curds of those infants who show casein masses in the stools with those who do not, it is evident that the former are larger and firmer. They are nevertheless always softer than the curds in the stool. But we have merely to shape these curds from the stomach, and to expose them for some hours to the air, to render them brown and at times almost indistinguishable from the bean-shaped curds of the stool. They are not so firm as the latter, probably because they have not undergone the continued pressure of the intestinal peristalsis. Apart from the difference in curd formation, nothing distinctive was noted in regard to the gastric contents of infants having casein masses in the stool. The stomach content was not exceptionally acid at any stage of digestion. This is what we should expect when we consider that rennin is inhibited by a high degree of acidity. Free hydrochloric acid was occasionally met with.

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[7] Tobler, L.: Ergeb d. inn Med., 1908, i.

It may be of interest to add a word concerning some attempts, although they proved negative, to increase the size or firmness of the gastric curd. Tobler[7] states that the curd can be kept for long periods in the stomach if the infant is fed very frequently, so that the solid matter does not gain an opportunity of passing the pylorus. This idea was carried out on three children who were fed every hour with about three to four ounces of milk. Although they had at some previous time passed curds, during this three days' test no curds were found in the stool. Infants fed by the cup did not react differently from those fed by the bottle, nor did those fed rapidly through a large opening in the nipple, differ in their stools from those in whom the food was given slowly through a small aperture.

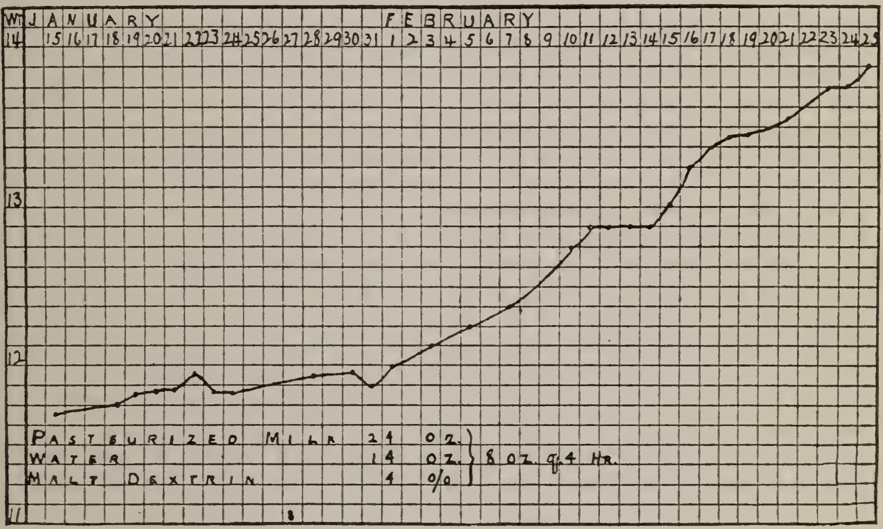

Chart showing steady gain in weight in spite of daily passage of large curds. I. L., aged 6 months.

Study of Duodenal Contents

So much for the formation of gastric curds. We next proceeded to study the duodenal contents of these children. Many hundreds of examinations by means of the duodenal catheter had given us a definite idea of the curds normally found in the duodenum in infants. These curds are typical, to such an extent indeed, that they have been of aid to us in judging whether the tube has passed the pylorus; they may be regurgitated from the duodenum into the stomach. We have always noted them in our records as *pyloric casts.* They vary in length from about ⅛ to ½ an inch, and give the appearance of having been compressed through a narrow channel. They are quite what we should expect when we reflect on the radiographic pictures of milk in its passage through the pylorus.[8] These curds afford us an opportunity of judging of the size and nature of the curd which normally enters the duodenum when an infant is fed on cow's milk. The duodenal contents of infants who passed the casein masses, especially those who passed these bodies constantly and in large numbers, was frequently found to be different. Although the diameter of the catheter prevented our obtaining a true picture of the size of the curds, it was noted that in these cases larger and coarser curds were aspirated, and that the lumen of the catheter became frequently plugged.

An examination of the trypsin was carried out in these cases. As the first step, the duodenal contents were aspirated from some infants who passed curds. It was found that the trypsin in these cases was normal, and was able to digest gelatin within twenty-four hours. It was just as potent as in infants who were not passing curds. Second, the curds from the stools were subjected to the action of the trypsin of the duodenal fluid of normal children, and it was found that even after they were kept under these conditions over twenty-four hours in the thermostat, the firm masses were not completely digested. In view of the fact that trypsin is one of the most powerful of the proteolytic ferments and readily digests casein, these tests show the importance of the density or compactness of a substance quite apart from its chemical constitution, in respect to its digestibility, and in particular of these casein masses. It should be considered, however, that these masses are probably much firmer when they are passed, than throughout their course in the small intestine.

A further experiment was instituted as a corrollary to these tests of trypsin. The curds of the stool were scraped, thoroughly washed with water, and then fed to some infants who passed curds, and to others who did not have them in the stools. Before feeding them, they were injected in the interior with fuchsin, so as to facilitate their recognition later. These curds were recovered in the stools from normal babies who for some time had not had casein masses in the stool. All of them were not found, but enough were recovered to show that these masses are incapable of being digested in the gastro-intestinal tract of the normal infant. Similar experiments with the gastric curds were undertaken, but thus far are not conclusive.

Occurrence of Curds and Effect on Health

Our series of investigations may be divided into two groups, both as regards time and method of approach. The first one taken a year ago consisted in noting throughout the asylum the various cases in which curds were found in the stool, and in keeping for months a daily record of these cases. This showed us that there were certain infants who passed these curds for long periods, although they seemed to be in perfect health, and no wise differing from others who were obtaining the same food and whose stools were normal.

More recently we have undertaken an experiment less broad, but more intensive in nature. We inspected the stools in a ward composed of sixteen infants, ten of whom were obtaining 40 ounces of ⅔ milk with 5 per cent.

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[8] These pyloric casts are frequently enveloped in a coat of mucus, which can be readily seen if the cast is floated on water, or in a fluid such as toluol, which makes the enveloping substance more opaque. When this is done, we frequently find a long tail of mucoid substance attached to the curd. These bodies may be similar to the food strings noted in the duodenum by Cannon by means of the fluoroscope. They are not formed merely in the passage through the catheter, as they cannot be obtained from the milk within the stomach, and are frequently of a diameter smaller than the catheter.

sugar, and who varied in age from 6 months to a year, and six infants varying in age from 1 to 2 years, who were receiving a pint of milk, together with mixed diet. The milk had been pasteurized at 147 F. for forty minutes. For a period of over a month, every stool was saved and inspected either by the house physician or by myself, and a record made as to its character and whether or not it contained casein masses. Vegetables were not given, as their coloring matter interfered with the examination of the stool. In the course of this month there was no child that did not at some time pass casein masses. Some passed them far more regularly than others; the younger group passed them more frequently than the older group. There was the same individuality noticed as had been remarked on last year; certain infants, independent of their well-being or of any clinical manifestation, had a peculiar tendency to pass bean-like masses in their stools. This tendency seemed to persist, for on examining the stools of the infants who passed curds so frequently last year, and who now are in the high-chair ward, it was found that many of them, an undue proportion, are still passing curds. The curds are not so large nor so hard in any instance as they were when the infant was younger. Incidentally, it may be of interest to note that in the course of these examinations of all the stools, it was most striking that certain infants have characteristic stools apart from the presence of curds, and that frequently the observer is able to designate the stool of a certain child by its appearance and odor, although the other children are on the same diet. It was also soon evident that it is impossible to draw a sharp distinction between casein and non-casein material, for there are gradations in the curds. Some, although small, soft, and easily broken up, are clearly, judging from their appearance, of the same constitution as the firmer and larger masses, and differ solely in having undergone partial digestion.

There is one more question which should be considered, namely, whether this disturbance is purely gastric in nature, or whether it is also dependent on some intestinal derangement. We have seen that in these cases there is no deficiency of trypsin. However, a lack of erepsin, the other intestinal ferment which is able to digest casein, would also favor the non-digestion of these curds and lead to their passage in the stool. The same would be true of any condition which increased intestinal peristalsis, although the secretion of ferments was normal. It can be definitely stated that these casein masses are found not only in normal stools, but in the stools of infants which have been normal for many consecutive days. They do not, therefore, viewed from the clinical standpoint, indicate an intestinal disturbance, nor do they by mechanical irritation lead to intestinal trouble. However, although there is no parallelism in this regard, when we look over the notes of about one thousand stools of the group of sixteen infants who were continuously observed, it is clear that the curds are found more often in stools that are not completely digested, and at times when there is some faulty digestion, than when the intestinal functions appear normal. They are noted most often when an infant had three or more stools in the course of twenty-four hours, but were not infrequently present when there was but one or two stools in the course of the day. Judged by the various clinical criteria, they seem to indicate simply non-digestion of abnormally curded casein, and not a specific type of indigestion,[9] for there may be a steady gain in weight and no signs of indigestion, no loss of appetite, no vomiting, no rise of temperature. It is significant in this regard that the infant which gained the most weight and with the greatest regularity during the month of observation, chanced to be the very one which had curds most regularly in the stools (Chart 1).

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[9] It was found that when 1 per cent. of powdered casein is added to the daily food of infants who pass these large curds, it does not result in the appearance in the stools of a larger number of the casein masses. If the disturbance were due simply to a lack of casein digestion or a casein indigestion, we should expect an increase of the bean-like masses. Viewed from the intestinal standpoint it is rather a casein-curd non-digestion. We cannot consider the digestive disturbance a "raw milk dyspepsia," as Ibraham has suggested, as it occurs so frequently when pasteurized milk is given.

CONCLUSIONS

From what has been stated, it would seem certain that the large bean-like masses in the stool concerning which there has been such difference of opinion, are curds which contain casein, and are formed in the stomach. The fact that they show no bile coloring matter in their interior goes to prove that they are moulded before they reach the level of the duodenum where the bile is poured into the intestine; the additional fact that they disappear when the food is introduced by catheter directly into the duodenum, and reappear when it is once more given by mouth, must be accepted as strong evidence towards this conclusion. We wish to lay emphasis on the observation that certain infants are peculiar in their tendency to pass the curds with slight intermissions for months, whereas other infants similarly fed pass stools normal in this respect. This shows that in studying the pathogenesis of this condition we must take a clinical as well as a laboratory point of view, and must consider the individual infant and not merely the chemical factors which favor or hinder the coagulation of milk in the stomach. There are some infants who produce larger and firmer curds in their stomachs, due either to the amount of rennin which they secrete, or to the abnormal conditions under which the enzyme acts on the milk. These larger masses were found in the stomach and also in the duodenum of infants who passed casein curds in the stools. Ordinarily, the milk curd is pressed from the pyloric curd of the stomach into the duodenum in the form of slender, compact " food strings," which may be termed *pyloric casts*. In the cases with curds in the stools, the catheter showed the duodenum to contain larger masses.

The curds are always of gastric origin and their pathogenesis is always gastric. The intestines play merely a secondary rôle. From the intestinal standpoint the curds indicate merely the non-digestion of casein masses. The intestinal disturbance which at times accompanies the appearance of these masses in the stool, may be of gastric origin, or of the same origin as the disturbed gastric function; it is not caused by the curds, nor is it the cause of the curd formation.

We cannot close without considering whether this clinical condition is harmful, and whether therefore, therapeutic measures should be taken to obviate the formation of these curds. Although a healthy infant may thrive in spite of the curds, we cannot infer that they may not sometimes lead to more serious disturbance. Furthermore, it is open to question whether foreign bodies of this size should be allowed to form and to traverse the intestinal tract. Diluting milk with barley water does not check their formation, nor does pasteurization to 147 F. for forty-five minutes, a process which complies with the ordinances of the health departments of various cities. Pasteurization to 170 or 175 F. causes the curds to disappear to a large extent, and therefore may be said to render the milk more digestible in these cases. We believe that the curds should not be allowed to persist, but should be checked, either by a high degree of pasteurization or by boiling the milk.

PRECIPITATION TESTS IN SYPHILIS

Peter K. Olitsky and Miriam P. Olmstead

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Wassermann, Neisser and Bruck applied Bordet-Gengou's phenomenon of complement fixation to syphilis in 1906. Since then, their principles have undergone many refinements until to-day the method of complement fixation stands out as a test *par excellence* for syphilis. The recognition of natural antisheep red cell amboceptor, the provisions against non-specific complement deviation, the use of inactive serum, the refinement of antigen, the cognizance of anticomplementary effects—all have determined the specificity of the Wassermann reaction.

Owing to the difficulties encountered in a reliable performance of the reaction, methods have been devised for the serodiagnosis of syphilis, having for their main objects simplicity and rapidity. These methods are based on precipitation in luetic serums by various reagents.

R. Kraus,[1] in 1897, discovered the precipitating action of immune serums by bacterial suspensions; but, as the *Treponema pallidum* is so difficult of cultivation, no similar method was used in syphilis to detect antibody. Fornet and Schereschewsky[2] modified this principle by adding the serums of tabetics (luetic) to those of earlier syphilis and obtained precipitations. They found the reaction specific and explained it by declaring that " antigen " existed in early syphilis serum and "antibody " in later. Several factors are concerned here that affect the specificity of this action which we shall show later.

Elias, Neubauer, Porges and Soloman[3] were the first to use a hydrophilic colloid. They employed a 1 per cent. solution of sodium glycocholate and obtained precipitation with syphilitic serums. These authors, however, have failed to demonstrate an absolute specificity in their reaction. Wieder and L'Engle[4] have used sodium taurocholate, taurin solution and distilled water, but while precipitation occurred there was a complete lack of specificity. Klausner[5] obtained a precipitum when fresh syphilitic serum was added to distilled water and allowed to incubate for several hours. Our experiences taught us that some non-luetic serums would give positive while some luetic serums would give negative results. Also, we confirmed Hayn's observation that inactivation as well as aging abolished the effect. Sachs[6] found that the addition of from 1:1,000 to 1:500 normal hydrochloric acid restores this property, but he, likewise, could not determine the specificity of the Klausner reaction. The effect of addition of the dilute hydrochloric acid is of interest in the Wassermann reaction, in which similar results may be had, and is probably due to the acid disturbing the hydroxyl concentration which occurs in inactive serums. Cuorin, used by Teruuchi and Toyada[7], and lecithin by Georg Meier[8] gave precipitation when added to syphilitic serums, but Meier showed that these precipitinogens had a high degree of non-specificity (50 per cent. of non-luetic serums gave precipitations).

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[1] Kraus, R.: Wien klin. Wchnschr., 1897, No. 32.

[2] Fornet and Schereschewsky: München. med. Wchnschr., 1907, No. 30, p. 1471.

[3] Elias, Neubauer, Porges and Soloman: Wien. klin. Wchnschr., 1908, No. 23.

[4] Wieder, Henry S., and L'Engle, Edward M : Some Studies of the Precipitation Tests for Syphilis, The Journal A. M. A., Nov. 6, 1909, p. 1535.

[5] Klausner: Wien. klin. Wchnschr., 1908, Nos. 7, 11, 13 and 16.

[6] Sachs: Semaine méd., June 24 1908

[7] Teruuchi and Toyada: Wien. klin. Wchnschr., 1908, No. 23.

[8] Meier: Berl. klin. Wchnschr., 1908, No, 10, p. 522,

Herman and Perutz[9] modified Elias, Neubauer, Porges and Soloman's reaction and asserted that a precipitation by their method meant syphilis. Remarkable attestations to this specificity have been appearing from then to date.

In 241 cases of syphilis, Thomsen and Boas[10] found the Wassermann reaction to be positive in 86 per cent., while the Herman-Perutz reaction was positive in 48.4 per cent. In 182 non-luetic cases the Wassermann was negative in all, the Herman-Perutz positive in three. One case was that of a third stage pulmonary tuberculosis, another of venereal ulcer without spirochetes and the third a case of osteomyelitis and sepsis with subsequent death, necropsy showing no syphilis. In 106 serums Jensen and Freilberg[11] found sixty-three non-luetic, with the Herman-Perutz reaction negative in all, and forty-three luetic with the Herman-Perutz reaction positive in all tested, corresponding to positive Wassermann reactions. Gammeltoft[12] found it a quick and convenient method for diagnosing syphilis. Of 156 control serums (negative) only one (cancer of the liver) gave a positive precipitation, in which case the Wassermann was negative. Ellermann,[13] Lade[14] and Kallós[15] (the observation of the latter two having been made recently) came to the same conclusions, that a precipitation means syphilis whenever it occurs.

We have investigated this question by applying this reaction 259 times to 175 serums. We employed the technic recommended by Herman and Perutz. This is, in short, as follows:

Two solutions are used:

Solution 1 (stock solution diluted 1:20 with distilled water before use):

Sodium glycocholate	2.0 gm.
Cholesterol	0.4 gm.
95 per cent. alcohol	100.0 gm.

Solution 2 (freshly prepared before use):
Sodium glycocholate 2 per cent. in distilled water.

The test was performed by adding to 0.4 c.c. of clear inactive serum (56 C. [100.8 F.] for half an hour) in a 7 mm. bore tube, 0.2 c.c. of Solution 1 and 0.2 c.c. of Solution 2. (We controlled the test by adding 0.4 c.c. of distilled water, instead of reagent, to the serums and for each set of tests adding 0.4 c.c. of distilled water to 0.4 c.c. of the reagent.) The tubes were plugged tightly with cotton and set aside at room temperature for twenty hours, when the presence or absence of precipitation was noted.

By this method Herman and Perutz obtained these comparative results: Of 89 non-syphilitic cases, only one reacted feebly (a serum from a tuberculosis patient), of 134 syphilitic serums, 102 were examined. The Wassermann reaction was positive in 72 and negative in 30, and their reaction positive in 76 and negative in 26.

In our series of examinations, the serums were all controlled by first performing the original Wassermann reaction, but using only one-tenth of Wassermann's amounts. Alcoholic extract of normal guinea-pig heart was employed as antigen, and fixation allowed for four hours in the ice-chest. Readings were made just as soon as the controls (containing twice the amount of antigen and serum used in the test) hemolyzed.

In 95 cases of syphilis in different stages we have found 88 giving a positive Wassermann reaction, 7 being negative. Correspondingly, the Herman-Perutz modification, when used, showed 58 positive, 3 doubtful and 34 negative reactions. In 80 non-luetic serums, the Wassermann reaction was negative in all, but the Herman-Perutz test was positive in 16. Of these, 50 per cent. were serums from chronic nephritics. Other positives were found in pulmonary tuberculosis, arterio-sclerosis, chronic arthritis, chronic endocarditis, obesity and a normal condition.

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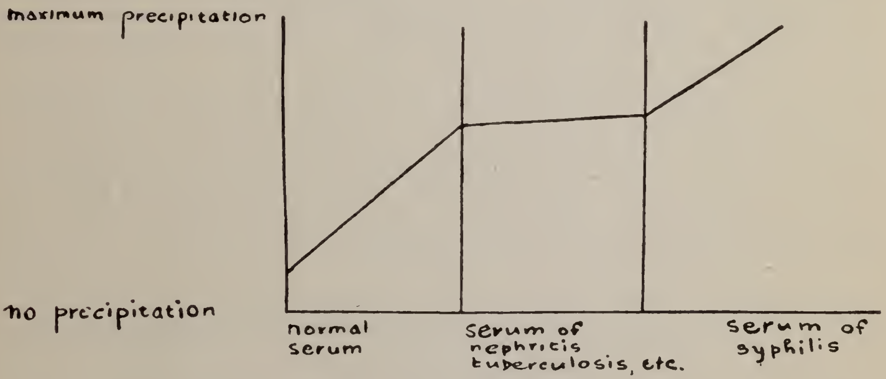
[9] Herman and Perutz: Med. Klinik, 1911, No. 2.
[10] Thomsen and Boas: Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1913, xvi, No. 4.
[11] Jensen and Freilberg: Berl. klin. Wchnschr., 1912, No. 23.
[12] Gammeltoft: Deutsch med. Wchnschr., 1912, No. 41.
[13] Ellermann: Deutsch. med. Wchnschr., 1912, No. 5.
[14] Lade: Deutsch. med. Wchnschr., 1913, No. 15.
[15] Kallós: Deutsch. med. Wchnschr., 1913, No. 39.

Strong[16] likewise found that 10 per cent. of non-specific serums gave a positive precipitation.

We attempted to obtain more reliable results by trying various modifications in technic. One series of serums was set aside in the ice-chest instead of being kept at room temperature, but no increase in precipitation was noted; two serums of fourteen, however, positive at room temperature, became negative in the ice-chest. Another series was incubated, but the results were far from satisfactory. This temperature offered a good field for the development of bacteria, molds, etc., which interfered with precipitations. Then again, great variations were found between the precipitinogenic Solution 1, when it was diluted by the addition of distilled water drop by drop, or when water was added *en masse*. Analogously to the results in the Wassermann reaction, many more positives were obtained by the former method. The results obtained by using active or inactive serums almost corresponded; but, since some of the positives obtained in active serums were non-specific and since these became negative in inactive serums, it appears best to use inactive serum under all circumstances. In fact, the modification of Herman-Perutz in all details gave the most constant results.

We attempted to substitute the precipitinogen by using an alcoholic extract of normal guinea-pig heart (Wassermann antigen), but there was a uniform precipitation in normal as well as luetic serums. This is due to the presence of alcohol, which has the power to coagulate the albumins present in serum. Others have obtained precipitations by using various fractions of syphilitic liver antigens, yet have not obtained a desideratum for the rapid diagnosis of syphilis.



Curve of precipitation of the Herman-Perutz reaction.

Rationale of Precipitation Reactions

We were struck by the constancy with which all serums, luetic or non-luetic, became clouded when these tests were performed. Indeed, only such as gave a distinct flocculation could be regarded as positive. This reaction is a colloidal one. There is a throwing of the sols into gel formation. It is represented by cloudiness of the serum. In fact, particles measuring 100 microns will give rise to haziness, yet the individual masses will be invisible, unless the ultramicroscope is called into play. The reactions are purely quantitative, not qualitative.

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[16] Strong: Jour. Med. Research, 1911, xxv, No. 1.

If, on an ordinate, we were to plot normal serum, serum from nephritis, tuberculosis or conditions in which cholesterol or the albumins have been found to be increased, and then syphilis; and on an abscissa we were to plot the degree of precipitation found by using the Herman-Perutz reaction, we should obtain a curve with its maximum point at syphilis and descending therefrom to normal serum, but never reaching normal serums at zero precipitation.

Other factors appear to interfere with precipitation tests. We have seen alcohol by itself in dilutions from 20 per cent. to 95 per cent. produce a gel formation. Again, serum is contaminated easily and, owing to consequent changes in reaction and surface tensions, spontaneous gel formation may occur. Elias, Neubauer, Porges and Soloman have shown that sodium glycocholate in 1 per cent. dilutions may give very light precipitations in normal serums.

Conclusions

The Herman-Perutz reaction or precipitation by hydrophilic colloids in general should be regarded as only partially specific. That is, only a heavy precipitation should be considered positive, and then only in a presumptive sense. A diagnosis of syphilis should not be made therefrom, but further investigations of such a serum is in order, especially by means of the Wassermann reaction.

The Wassermann reaction is by far the more reliable of the two.

There is no absolute parallelism between precipitation and complement fixation. The degrees of similarity should not warrant the theory that antigen precipitates antibody, thus binding complement.

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COMPLEMENT FIXATION IN PERTUSSIS

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Since Bordet and Gengou in 1906[1] announced the discovery of the etiological cause of whooping cough, basing their claim mainly on the complement fixation reactions given by the serum of whooping cough convalescents, complement fixation has been studied by many serologists, both from an etiological and from a diagnostic standpoint.

Bordet and Gengou used as antigen a saline emulsion of a culture of the Bordet-Gengou bacillus on solid medium. Four hours at room temperature were allowed for fixation, and readings were made the following day. In later publications[2] the constancy of a positive complement fixation reaction among convalescents is reaffirmed, but no figures are given. The work of Bordet and Gengou has been confirmed more or less completely by some investigators and by others their claim has been declared invalid. Meier[3] found the serum of pertussis patients to react with an extract of the lung tissue of patients dying of pertussis. Arnheim[4] using an antigen of the Bordet-Gengou bacillus, obtained positive reactions in six out of twelve cases and thought imperfect technic might account for the lack of a positive reaction in the other cases. In a later publication[5] he stated that these twelve cases were tested again and also three others, and that by making the readings immediately instead of on the following day, as before, he obtained a positive reaction in twelve of the fifteen. The antigen consisted of an aqueous emulsion heated at 58 to 60 C. for one hour, shaken on the following day for three hours and centrifuged. Fraenkel[6] tested the serum of five pertussis convalescents against an antigen of the Bordet-Gengou bacillus and obtained a positive reaction in only one, though his technic was apparently the same as Bordet's. Seiffert[7] mentions a positive complement fixation reaction given by one case of whooping cough, against an antigen of the Bordet-Gengou bacillus. Klimenko[8] has reported one case, convalescent three weeks, that gave a positive reaction with a Bordet-Gengou antigen. Wollstein[9] tested the serum from nine patients, in the first to the ninth week of the disease, against antigens of the Bordet-Gengou bacillus and the influenza bacillus, and all results were negative. She used as antigens salt solution suspensions of the Bordet-Gengou bacillus prepared according to the method of Bordet and Gengou, extracts of the bacilli, made by suspending the growth of three blood-agar slants in 5 c. c. of salt solution and shaking twenty-four hours in the thermostat, and extracts from the lungs of a case that came to necropsy, prepared according to the method of Wassermann and Meier. Menschikoff[10] reported positive results with the serum of two pertussis convalescents, using antigens of the Bordet-Gengou bacillus. Later Baecher and Menschikoff[11] found the serums of whooping cough cases examined in the first to the sixth week of the paroxysmal stage, to have no complement fixation power; but positive reactions were obtained with the serums of cases repeatedly vaccinated. Four hours at room temperature were considered necessary for the fixation of complement. The antigen used was a saline emulsion of Bordet-Gengou bacilli. Twenty-one treated cases were tested, nine of which gave a positive reaction. Finizio[12] tested the serum of eight convalescents and obtained six positive reactions with an antigen of the Bordet-Gengou bacillus. Poleff[13] tested two cases in a late stage of whooping cough, using as antigen a saline suspension of two strains of the Bordet-Gengou bacillus; and the results were negative. Imai[14] in testing the blood of convalescents by complement fixation confirmed the results of Bordet and Gengou.

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[1] Bordet and Gengou: Le microbe de la coqueluche, Ann. de l'Inst. Pasteur, 1906, xx, 731.

[2] Bordet and Gengou: Note Complémentaire, Ann. de l'Inst. Pasteur. 1907, xxi, 720; Note complémentaire sur le microbe de la coqueluche et sa variabilité au point de vue du serodiagnostic et de la toxicité, Centralbl. f. Bakteriol., orig., 1912, lxvi, 276.

[3] Meier, G.: Anwendung der Komplementbindungsmethode bei Keuchhusten, Deutsch. Med. Wchnschr., 1907, xxxiii, 1558.

[4] Arnheim, G.: Ueber den gegenwartigen Stand der Keuchhusten Frage, Berl. klin. Wchnschr., 1908, xlv, 1453.

[5] Arnheim, G.: Keuchhustenuntersuchungen, Arch. f. Kinderh., 1909, 1. 296.

[6] Fraenkel, C.: Untersuchungen zur Entstehung des Keuchhustens, München. med. Wchnschr., 1908. lv, 1683.

[7] Seiffert, G.: Ueber den Bordetschen Keuchhustenbazillus, München. med. Wchnschr., 1909, lvi, 131.

[8] Klimenko, W. N.: Die Aetiologie des Keuchhustens, Centralbl. f. Bakteriol., orig., 1909, xlviii, 64.

[9] Wollstein, M.: The Bordet-Gengou Bacillus of Pertussis, Jour. Exper. Med., 1909, xi, 41.

[10] Menschikoff, W.: Ueber den Erreger des Keuchhustens, Russk. Vrach., 1909, p. 1044.

Bordet and Gengou[15] in 1911 called attention to the value of complement fixation in diagnosing cases of pertussis without a whoop or other typical symptom and mentioned one such case, an adult. Gengou and Brunard[16] had previously reported positive complement fixation reactions with the serum of three adults who had been coughing for two months and who undoubtedly had whooping cough. Delcourt[17] had likewise demonstrated the diagnostic value of complement fixation by obtaining positive reactions in seven doubtful cases of pertussis. Netter and Weil,[18] working with the Bordet-Gengou bacillus, found complement fixing substances to develop too late to be of service in the diagnosis of whooping cough in normal cases. With one exception (a child that had been whooping six days) they obtained no positive reactions even by the use of active serum earlier than the second week of the whoop. During the second week active serum was found to give more positive reactions than inactive. After the fifteenth day all sixteen cases tested reacted positively, whether active or inactive serum was used. In regard to the duration of the complement fixation reaction, Weil[19] claimed that a positive reaction was always given within three months of cure and might be given thirteen years after cure. He based this statement on a study of nine patients who had been cured for from two months to thirteen years. Weil suggests that other diseases, for example, measles, may have an effect on the complement fixation reaction in pertussis. Of three cases of measles that had been whooping three to five weeks, he found two to give a negative complement fixation reaction with a Bordet-Gengou antigen and the other one to give only a weakly positive reaction. Friedlander and Wagner[20] have reported positive complement fixation results in all stages of pertussis, including the early catarrhal, and consider the test of great diagnostic value. Their antigen is a saline emulsion of a seventy-two hour growth of the Bordet-Gengou bacillus on ascitic fluid agar. They have used active serum. A water bath at 37 C. was used for fixation and incubation and readings were made within an hour after the addition of cells and amboceptor. Friedlander and Wagner obtained a positive complement fixation test in all eighteen cases tested in the paroxysmal stage. Of twelve cases in the catarrhal stage, from one to five weeks before the appearance of the whoop, eleven gave positive reactions. One case of doubtful pertussis gave a positive reaction. Sixteen normal individuals were negative.

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[11] Baecher and Menschikoff: Ueber die Aetiologische Bedeutung des Bordetschen Keuchhustenbacillus und den Versuch einer spezifischen Therapie, Centralbl. f. Bakteriol., orig., 1911, lxi 218.

[12] Finizio, G.: Der Bordet-Gengousche Bacillus in der Aetiologie des Keuchhusten, Ztschr. f. Kinderh., orig., 1911, iii, 121.

[13] Poleff, L.: Ueber den Bordet-Gengouschen Keuchhustenbacillus, Centralbl. f. Bakteriol., orig., 1913, lxix, 23.

[14] Shiga, Imai and Eguchi: Eine Modification von Bordet-Gengous Nahrboden für die Keuchhustenbacillen nebst einigen Ergebnissen in serologischer Beziehung, Centralbl. f. Bakteriol., orig., 1913, lxix. 104.

[15] Bordet and Gengou: Le diagnostic de la coqueluche fruste par la methode de la fixation d'alexine, Centralbl. f. Bakteriol., orig., 1911, lviii, 573.

[16] Gengou and Brunard: Apropos du diagnostic de la coqueluche chez l'adulte, Bull. de l'Acad. Roy de méd. de Belgique, 1910, xxiv, 329.

[17] Delcourt: Diagnostic de la coqueluche fruste par la Réaction de Bordet-Gengou, Arch. de méd. d. enfants, 1911, xiv, 30.

[18] Netter and Weil: La déviation du complément par le bacille de Bordet-Gengou dans la coqueluche, Comp. rend. Soc. de biol., 1913, lxxiv, 236.

[19] Wei. M.: La déviation du complément vis-à-vis du bacille de Bordet-Gengou dans la coqueluche, Compt. rend. Soc. de biol., 1913, lxxiv, 260.

[20] Friedlander and Wagner: Diagnosis of Whooping Cough by the Complement-Deviation Test. Jour. Am. Med. Assn., 1914, lxii, 1908; Am. Jour. Dis Child., 1914, viii, 134.

The Bordet-Gengou bacillus is not the only one that has been found to give a positive complement fixation reaction with the serum of pertussis cases. Manicatide,[21] who claims to have discovered in *Bacillus Z.* the etiological factor in pertussis, has found the serum of nineteen whooping cough cases to give a positive complement fixation reaction with an antigen of *Bacillus Z.* Negative reactions were given by six normals.

The variation in the results of different workers may be explained partly by differences in technic, such as, method of preparing antigen, amount of antigen used in test, amount of serum used in test, use of active or inactive serum, time and temperature allowed for fixation, interval between the addition of sensitized cells and the reading of results, method of reading reactions, partly by a difference in the interval between taking and testing the blood specimens, and partly by the small number of cases tested. An explanation of some discrepancies is still to be found. It is difficult to reconcile Manicatide's findings with those of Bordet and his followers.

The objects of our investigation were to test the validity of the claim for the etiological relationship of the Bordet-Gengou bacillus to whooping cough and to determine the diagnostic value of complement fixation in this disease. Various strains of the Bordet-Gengou bacillus, of atypical Bordet-Gengou bacilli and of hemoglobinophilic bacilli have been classified according to their complement fixation reactions by elaborate cross titrations of immune rabbit serum and this work is soon to be described in another article.

The strains[22] used for antigens in the tests with human serum are described in Table 1. The typical Bordet-Gengou bacilli may, according to their reactions with immune rabbit serum, be considered practically identical, closely related to the hemoglobinophilic bacilli but distinct from them. The atypical Bordet-Gengou bacilli are (with the exception of C) more or less closely related to each other and to the typical Bordet-Gengou bacillus. The hemoglobinophilic strains are more or less closely related to each other.

Antigens have been prepared by Dr. O. Povitzky as follows: For the four atypical Bordet-Gengou strains, which grow on plain veal agar, Dr. McNeil's method of preparing gonococcus antigen has been successfully employed. Twenty-four hour cultures on salt free veal agar are washed off with sterile neutral distilled water, the emulsion is heated in a water bath at 56 C. for three hours, and at 80 C. for one hour and filtered through a Berkefeld filter. Influenza antigens are prepared from a twenty-four to forty-eight hour growth on 1 to 500 blood veal agar, washed off with sterile neutral distilled water, shaken for two to four hours, left in a thermostat at 56 C. for about eighteen hours and centrifuged or filtered through a Berkefeld filter. Antigens are prepared from the typical Bordet-Gengou

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[21] Manicatide: Der Komplementbindungsvorgang bei Keuchhusten, Ztschr. f. Kinderh., 1913, vii, 226.

[22] Most of the strains were isolated by Dr. A. W. Williams and Miss M. A. Wilson and the morphological and cultural identification was made by them. (Williams, A. W.: The Etiology of Pertussis, Arch. Pediat., 1914, xxxi, 567.)

TABLE 1.

STRAINS USED FOR ANTIGENS.

Strain	History	Characteristics		
		Morphological	Cultural	Serological
P. D.	From another laboratory, originally from Prof. Bordet	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
55	From sputum of a case in the sixth day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
81	From sputum of a very severe case in the fifth day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
93	From a throat swab of a case in the eighth day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
95	From sputum of a very severe case in the first day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
98	From sputum of a case not whooping but developing whoop later	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
100	From sputum of a case in the third day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
114	From sputum of a case with doubtful whoop	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
121	From sputum of a case in the first day of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
141	From sputum of a case in the second week of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
154	From sputum of a case in the second week of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
155	From sputum of a case in the third week of the paroxysmal stage	Typical Bordet-Gengou	Typical Bordet-Gengou	Fixation with all other Bordet-Gengou strains
C.	Probably from pertussis, isolated about two years	Atypical Bordet-Gengou	Atypical Bordet-Gengou. Grows without blood	No cross fixation.
1	From sputum isolated about one year; no record of source	Atypical Bordet-Gengou	Atypical Bordet-Gengou. Grows without blood	Some cross fixation with Bordet-Gengou strains
31	From sputum of case in third week of paroxysmal stage	Atypical Bordet-Gengou	Atypical Bordet-Gengou. Grows without blood	Some cross fixation with Bordet-Gengou strains and with 10
10	From sputum of case in third week of paroxysmal stage	Atypical Bordet-Gengou	Atypical Bordet-Gengou. Grows without blood	Some cross fixation with 31
33	From sputum of case in second week of paroxysmal stage	Typical influenza bacillus.	Hemoglobinophilic bacillus	Fixation with some other influenza strains
35	From sputum of case in fourth week of paroxysmal stage	Typical influenza bacillus.	Hemoglobinophilic bacillus	Fixation with some other influenza strains
37	From sputum of case in second week of paroxysmal stage	Typical influenza bacillus.	Hemoglobinophilic bacillus	Fixation with some other influenza strains
87	From sputum of case in first week of paroxysmal stage	Typical influenza bacillus.	Grew several generations on ascitic, then became hemoglobinophilic	Fixation with some other influenza strains
747	From spinal fluid of case of cerebrospinal meningitis	Typical influenza bacillus.	Hemoglobinophilic bacillus	Fixation with some other influenza strains
BI$_2$	From another laboratory	Typical influenza bacillus.	Hemoglobinophilic bacillus	Fixation with some other influenza strains
Z	From spinal fluid of case of cerebrospinal meningitis	Typical influenza bacillus.	Probably hemoglobinophilic bacillus	Fixation with some other influenza strains

strains in the same manner except that Bordet-Gengou potato-blood-agar medium is used and the growth, instead of being washed off, is scraped off with a platinum spud and deposited in the distilled water, in order to avoid extraction from the medium of substances that would render the antigen anticomplementary and nonspecific.

Inaba's[23] antigen, prepared by emulsifying three loops of a forty-eight hour culture in 5 c. c. of physiological salt solution, shaking one to two hours for two days and centrifuging, has been tried but has proved to be no more satisfactory than our antigens; that is, no more specific and of no more value in reacting with the serum of pertussis cases. The antigen described by Friedlander and Wagner,[24] a saline emulsion of a seventy-two hour growth on ascitic agar, has also been tried. The tests on whooping cough cases have been too few to be conclusive, but thus far this antigen has given no more positive reactions than ours and has seemed to be less specific. It has given strong + (doubtful) reactions with serums from normal adults, and it has cross-fixed with the serums of rabbits immunized against atypical Bordet-Gengou strains and influenza strains. Furthermore, it has the disadvantages of all live culture antigens: it must be fresh and it must be standardized immediately before use.

Antigens are standardized at the temperature to be used for tests by determining the antigen unit with a homologous immune serum and the anticomplementary dose of the antigen. For tests one-quarter the anticomplementary dose is chosen, provided that amount gives complete inhibition with the homologous serum; and the antigen is diluted so that 0.1 c. c. contains the desired amount. Best results, strongest and most specific, are obtained with an antigen of long range. An antigen of short range has occasionally been used in an amount greater than one-quarter the anticomplementary dose, but the results are likely to be unsatisfactory.

For a hemolytic system we have used sheep cells in a 5 per cent. suspension, rabbit amboceptor and guinea pig complement in a 10 per cent. dilution. The system is carefully standardized daily by means of an amboceptor titration and for tests between two and three units of amboceptor are employed. The erythrocyte suspension is sensitized before use. The size of the test is one-tenth that of the classical Wassermann, 0.5 c. c. instead of 5 c. c.

The patient's serum is tested in two amounts, 0.02 c. c. and 0.01 c. c. and the anticomplementary property of the serum is determined by 0.04 c. c. and 0.02 c. c. controls. All specimens have been tested inactive and a few comparative tests have been made with active serum.

For fixation a water bath or incubator at 37 C. has been used for some tests on all specimens, and the results of these tests only are given in the accompanying tables. A few comparative tests have been made at room temperature for four hours and at ice-box temperature for four hours. For the second incubation, the water bath is invariably used and the tests are read as soon as the controls, double the amount of antigen used with the serum and double the maximum amount of serum used, are completely hemolyzed. Citron's[25] standard of readings is followed and only those tests are considered positive in which 0.02 c. c. of serum gives complete inhibition of hemolysis.

Tests have been made on specimens of serum from one hundred eleven pertussis cases or suspected pertussis cases (table 5). Nineteen of these specimens were received when no Bordet-Gengou antigens were on hand and so were tested against atypical Bordet-Gengou strains and hemoglobinophilic strains only. With these antigens no serum, inactive, gave a positive reaction. Of eleven specimens tested active, three gave a positive reaction with strain C and one with strain BI_2.

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[23] Inaba, I.: Ueber den Bordet-Gengouschen Keuchhustenbacillus, Ztschr. f. Kinderh., 1912, iv, 252.

[24] Friedlander and Wagner: Diagnosis of Whooping Cough by the Complement-Deviation Test, Am. Jour. Dis. Child., 1914, viii, 134.

[25] Citron: Immunity. Translated by Garbart., 1914, p. 184.

Tables 2, 3 and 4 contain the results of tests by water bath or incubator fixation on the inactivated serum of the ninety-two specimens that were tested against Bordet-Gengou antigens. The specimens are divided into three groups, namely, those from unvaccinated cases of pertussis (Table 2), those from vaccinated cases of pertussis (Table 3), and those from prophylactic cases, *i. e.,* cases that received vaccine before or after exposure to whooping cough and that did not develop a whoop (Table 4). Forty-eight unvaccinated cases were tested, thirty-two in the first to the seventeenth week of the paroxysmal stage and sixteen in the first to the twenty-first week of convalescence. Twelve of the whooping cases gave a postive reaction with at least one Bordet-Gengou antigen, a percentage of 37.5; and two of the convalescents gave a positive reaction, a percentage of 12.5. The earliest case tested had been whooping two days. The earliest case that gave a positive reaction had been whooping five days. The total number of vaccinated cases tested was thirty-two, six in the fourth to the seventh week of the paroxysmal stage and twenty-six in the first to the twelfth week of convalescence. Three of the whooping cases gave a positive reaction with at least one Bordet-Gengou antigen, a percentage of 50, and fifteen of the convalescent cases, a percentage of 57.7. The highest percentage of positives among unvaccinated cases has been obtained in the paroxysmal stage, among vaccinated cases the highest percentage occurred after the cessation of the whoop. The number of cases is insufficient, however, to establish this as a rule. The fact that the convalescent vaccinated cases were tested within the first three months of convalescence and most of the convalescent unvaccinated later, should be taken into consideration in comparing the two series (Table 6).

Two unvaccinated cases (7 and 14) were tested at two different times. The serum of Case 7 was on both occasions positive with the Bordet-Gengou antigen P. D. Case 14 gave a ± reaction the first time, a +++ reaction the second. This probably does not indicate a development of complement fixing substances between the first and the second bleedings, in the fifth and the seventh week, respectively, of the paroxysmal stage, but is due to the fact that the first specimen was not tested promptly and had lost antibody content when the test was made. This is the only specimen that, inactive, gave a positive reaction with antigen C; forty-seven were tested. The second specimen of Case 14 (14b) gave a positive reaction with Antigen 1; only one other specimen (110) out of twenty-nine tested against Antigen 1 gave a positive reaction with this strain. Cases 7b and 14b were the only specimens out of nine to give a positive reaction with the hemoglobinophilic strain 35. Case 14b also gave a positive reaction with BI_2, which appears to be less closely related than any other hemoglobinophilic strain studied to the Bordet-Gengou bacillus; tests on thirty-five other specimens from pertussis cases were negative or doubtful. Whether these reactions of 7 and 14 were nonspecific, due to undeveloped technic, or the poor antigens with which the work was begun, or whether they were due to a mixed infection in the cases, is not known. All tests with antigens 31, 10, 33, 37, 747 and Z have been negative or doubtful. The positive reactions given by antigen 87 are of special interest, as this strain was obtained from the sputum of a pertussis case. Of twenty-seven specimens tested against this

TABLE 2.

Complement Fixation Reactions of Forty-Eight Inactive Specimens from Forty-Six Unvaccinated Cases of Pertussis.

Serial Number	Stage of Disease	Complications	Antigens Used																						
			P. D	55	81	93	95	98	100	114	121	141	154	155	C	1	31	10	33	35	37	87	747	BI?	Z
7a	Whooping 36 days		+ + +												−									−	
7b	Whooping 59 days		+ + +												−	+	−			+ +				−	
11a	Whooping 102 days		±												+ + +	−	−							+	
14b	Whooping 117 days		+ + +												+	+ +	+			+ + +				+ +	
16	Whooping 66 days		−												+	−	−								
17	Whooping 108 days	Congenital idiocy	−												−	−	−							−	
18	Whooping 102 days		−												−	−	−							−	
26	Whooping 81 days		±												−	−	−			−				−	
27	Whooping 35 days		−												−	−	−		−	−				−	
28	Whooping 90 days		±													−	−							+	
30	Whooping 49 days		±												−	−	−		−	−				−	
31	Whooping 60 days		±												−	−	−		−	−				−	
32	Whooping 21 days		+ +																	−					
33	Whooping 24 days		+ +																					−	
34	Whooping 14 days		+ +																						
35	Whooping 60 days		±						+ + + +																
39	Convalescent 5 mos.		±	±	−	−	−	−	−													+ + +	−	−	
40	Convalescent 3 mos., 7 days		±	+		−	±	±	−													±		−	
41	Convalescent 3 mos.		−	−	−	−			−													+		−	
42	Convalescent 4 mos.,12 days		±	±	−	−			−													+ + +		−	
54	Convalescent 2 mos.		−																						
55	Convalescent 4 mos., 6 days		−	±		−																			
56	Convalescent 104 days		−	±		−																			
57	Convalescent 112 days		−	−		−	−																−		
58	Convalescent 104 days		−	−	−	−	−															+			
59	Convalescent 143 days		±	+	−	−	−															+ + +			
60*	Convalescent 4 mos		−	−		−																			
62	Convalescent 5 mos.		+ + +	+ + +	+ +	+	±	+ + +														+	−		
63	Convalescent 4 mos		±																						
76	Whooping 4 days	Measles	−					−																	−
77	Whooping 15 days	Measles	−												±			−							±
78	Whooping 2 days	Measles	−												±			±							±
79	Whooping 11 days	Measles	−												−										−
80	Whooping 46 days	Scarlet fever	±												±			−							−
81	Whooping 11 days	Scarlet fever	−												±			±							−
82†	Whooping 3 days		−							−					−			±							−
83	Whooping 2 wks.				±												−	−				−			
84	Whooping 21 days				+			±				±			±		−	−							
95	Whooping 5 wks.	Scarlet fever, chickenpox, otitis media			+ + +													±						−	
96	Whooping 30 days	Broncho-pneumonia			+ + +													+						−	
97	Whooping 3 wks				+													−							
98	Whooping 14 days				+ + +													−						−	
99	Whooping 4 wks.	Tubercular glands			+													±						−	
100	Whooping 5 wks				+	+ +		+			+ +			±	−	−		−		−		±	−		
101	Convalescent 5 days				+	+ +		+			+ +			+	−	−		−		±		±	−		
104	Whooping 5 days			+ + +	+ + +	+ + +		+ + +			+ + +			+ + +	−	−	+	−			−	−			
105	Whooping 2 mos.	Diphtheria; otitis media		+	−	±		−					−		−	−	−					±			
106	Whooping 23 days			+ + +	±	+		±					+ + +		−	±	−					−			
108	Convalescent 2 wks	Diphtheria					+	+						+		±						±			
109	Convalescent 1 wk.	Pneumonia					−	−					−	−		−						±			

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* Strain 55 was obtained from this case.

† Strain 141 was obtained from this case.

TABLE 3.

COMPLEMENT FIXATION REACTIONS OF INACTIVE SERUM FROM TWENTY-NINE VACCINATED CASES OF PERTUSSIS

Serial Number	Stage of Disease	Complications	Vaccine Administered§	Amount Vaccine in Millions	Interval Between Last Vaccination and Complement Fixation Test	Antigen Used																				
						P. D.	55	81	93	95	98	100	114	121	141	151	155	C	I	31	10	37	87	747	BB	Z
13	Whooping 12 days		P. D.	200	1 mo.	−												−	−	−					−	
29	Whooping 21 days		P. D.	100	10 days	+														+					+	
37	Convalescent 56 days	Chronic bronchitis	1	860	34 days	+ + + +	+ + + +	+ +	+ + +			+ + +											±		−	
38	Convalescent 56 days		1	1,860	31 days	−	−					−														
43	Convalescent 53 days		1 2	10,000 1,500	48 days	+	+ +		+	±		±											+ + +		−	
44	Convalescent 83 days		1	875	86 days	−	−	−	−		−												+		−	
46	Convalescent 83 days		1	875	84 days	±	+		±	−		−														
47	Convalescent 55 days	Tonsillitis	1	2,400	53 days	+ + + +	+ + + +		+ + + +																	
48	Convalescent 85 days		1	825	86 days	+	+ + +		−																	
49*	Convalescent 47 days		1 2	12,500 2,500	39 days	−	±	−	−	−													+		−	
50	Convalescent 50 days		1	8,000	50 days	+ + + +	+ + + +		+ + +	+ + +																
51	Convalescent 52 days		1	4,150	53 days	+ + +	+ + + +		−																	
52	Convalescent 36 days		1 2	66,500 11,000	38 days	+ + +	+ + + +		−																	
53	Convalescent 31 days		1 2	750 8,500	30 days	+ + +	+ + + +		±	±																
61	Convalescent 57 days		1	4,850	57 days	−	−			−														−		
64†	Convalescent 20 days		1 2	100 18,700	10 days	+ + + +	+ + + +			+ + +		+ + + +											+	−		
67	Convalescent 36 days		1 2	4,350 1,000	36 days	+ + +	+ + +			−													±			
68	Convalescent 17 days		1 2	5,350 2,000	36 days	−	−			−													+ + +	−		
72‡	Convalescent 36 days		1 2	3,850 6,000	34 days	±	±	±		−	±													−		
85	Convalescent 2 wks		3					+ + +			±		−		+			−		−	−					
86	Convalescent 1 wk		3					+ + +			+		−		+			−		±	±					
87	Convalescent 1 wk		P. D.					±			+				−					−	−					
88	Convalescent 19 days		1 3					±																		
90	Convalescent 1 wk		3					+ + +			+							±		−	−				±	
91	Convalescent 1 mo.		5					+			±							−		−	−				+	
93	Convalescent 13 days		3					+ +			±									−	−				−	
94	Convalescent 11 wks.		3					+ + +			±							−		−					+	
102	Whooping 27 days	Broncho pneumonia							+ + +														−			
103	Whooping 25 days	Broncho pneumonia	6				+ + +	+ + +	+ + +		+ + +			+ + +			+	−	−	−	−	−	−			
107	Convalescent 1 wk		8	250						+	±					−	+		±				±			
110	Whooping 5 wks.	Pneumonia	8	1,975						+ + + +	+ + + +					±	+ + + +		+ + + +				−			
111	Whooping 3½ wks.		8	750						+	±						+						−			

*Strain 87 was obtained from this case.
†Strain 100 was obtained from this case.
‡Strain 98 was obtained from this case.

§ Vaccine 1—Strains P. D., 33, 35, 37. Vaccine 2—Strains P. D., C, I. Vaccine 3—Strains 55, 93, 98, 100. Vaccine 5—Autogenous. Vaccine 6—Strains 55, 93, 95, 98. Vaccine 7—Strains 55, 93, 95, 98, differs from Vaccine 6 in method of preparation. Vaccine 8—Strains 154, 155, 163.

TABLE 4.

Complement Fixation Reactions of Inactive Serum from Twelve Vaccinated Cases Which Did Not Develop Into Typical Pertussis.

Serial Number	Symptoms	Vaccine Administered	Amount Vaccine in Millions	Interval Between First Vaccination and Complement Fixation Test	Antigens Used												
					P. D.	55	81	93	95	98	100	C	31	10	87	747	BI_2
36	Slight cough	1	1,040	50 days	±	+	....	±	+	....	—	....	....	....	+	....	—
45	Cough	1	850	2 mos.	—	—	....	....	—	....	....	....	....	....	+	—	—
65	Occasional cough	1	1,750	39 days	—	....	....	....	....	....	....	....	....	....	....	....	....
66	None	1	650	57 days	—	—	....	....	—	....	....	....	....	....	....	—	....
69	Cough for two weeks	1	4,350														
		2	1,000	43 days	+++	++	....	....	—	....	....	....	....	....	....	—	....
70	Bronchitis	1	3,350														
		2	2,500	36 days	±	—	....	....	....	....	....	....	....	....	....	....	....
71	None	1	850	57 days	+	—	....	....	—	....	....	....	....	....	....	—	....
73	Cough two weeks	1	4,350														
		2	2,500	36 days	±	—	....	....	—	....	....	....	....	....	....	—	....
74	Slight cough	1	650	57 days	—	—	....	....	—	....	....	....	....	....	±	....	....
75	Slight cough	1	650	43 days	—	—	....	....	—	....	....	....	....	....	+	....	....
89	Cough	3	....	....	......	....	±	....	....	—	....	....	±	—	....	....	+
92	Cough	5	....	....	......	....	+	....	....	—	....	—	—	—	....	....	+

strain, five (18.5 per cent.) have given positive reactions, and one of these (43) also gave a positive reaction with the Bordet-Gengou strain 55. The total number of specimens tested against antigens of hemoglobinophilic strains is sixty; the total number giving a positive reaction is six.

There is a marked lack of uniformity among the reactions given by different Bordet-Gengou strains with the same serum. This may be partly due to an inherent difference in the strains that has not been apparent in the cross-titrations; this point is to be investigated later. One reason is a difference in the range of the antigens; the longer the range of an antigen, the more likely it is to give the maximum number of specific fixations. Another reason for the lack of uniformity, unfortunately only recently observed, is the instability of an antigen after the addition of the salt. The aqueous extract antigens that have been used are stable for many months, but after being made isotonic they lose fixing power and cannot be depended on for more than two or three days. This fact undoubtedly accounts for some of the negative and doubtful reactions among convalescent cases and those late in the paroxysmal stage. Some serologists would probably consider positive many of the + reactions, those in which there is only slight hemolysis in the tube containing 0.02 c. c. of serum. Many of these reactions undoubtedly are specific; but inasmuch as a reaction of the same strength may be given by an antigen of a closely allied organism that is not the infecting agent, it seems wise to hold to the rule of calling only ++, +++ and ++++ reactions positive. Only cases of pertussis have given ++, +++ and ++++ reactions. The presence of natural antisheep amboceptor in the patients' serum has not been taken into consideration and may account for some doubtful and negative reactions.

TABLE 5.

SUMMARY OF COMPLEMENT FIXATION REACTIONS AMONG 111 CASES OF PERTUSSIS INCLUDING TWELVE DOUBTFUL CASES DESIGNATED AS PROPHYLACTIC.

		Number Positive	Percentage Positive
Number cases tested	111		
Cases not tested against Bordet-Gengou antigens	19		
Cases tested against Bordet-Gengou antigens	92	33	35.9
1. Vaccinated cases of pertussis	32	18	56.2
Vaccinated cases whooping	6	3	50.0
Vaccinated cases convalescent	26	15	57.7
2. Not vaccinated cases of pertussis	48	14	29.2
Not vaccinated cases whooping	32	12	37.5
Not vaccinated cases convalescent	16	2	12.5
3 Doubtful cases of pertussis—vaccinated	12	1	8.3

Complicating infections, such as measles and scarlet fever, may, as suggested by Weil,[19] cause a weakening of the complement fixation reaction for whooping cough. We have tested only four cases suffering from both measles and pertussis. The four all gave negative reactions with antigen P. D., but as they had been whooping only two, four, eleven and fifteen days, respectively, this result was not to be wondered at. The three scarlet fever cases had been whooping one, five and six weeks, respectively, and the second gave a positive reaction with antigen 81. Owing to the low percentage of positive reactions among cases of pertussis without complications, these tests prove nothing as to the effect of other infections on the antibodies of the Bordet-Gengou bacillus.

Among sixty-seven specimens of serum from normals or from patients suffering from some disease other than pertussis (chiefly syphilis or gonococcus infection), no positive reaction has been given by inactive serum with a Bordet-Gengou or an atypical Bordet-Gengou antigen. Tests with influenza antigens have also been negative, with the exception of two positive reactions with BI_2 and one with 87. As an influenza infection in these three individuals prior to the taking of the blood specimens could not be excluded, there was no proof of the nonspecificity of the reactions.

TABLE 6.

COMPLEMENT FIXATION REACTIONS AMONG SIXTEEN UNVACCINATED AND TWENTY-SIX VACCINATED CONVALESCENT CASES OF PERTUSSIS.*

Weeks of Convalescence	Not Vaccinated (16 Cases)	Vaccinated (26 Cases)
1	+−	++−−
2	−	++
3	....	+−
4	....	+−
5	....	++−
6	....	−−
7	....	+++
8	−	++−−
11	....	+
12	−	+−−
13	−	
15	−−	
16	−−−	
17	−	
18	−	
20	+−−	

* Each + symbol denotes one case having positive reaction. Each − symbol denotes one case not having positive reaction.

Total not vaccinated = positive 2 or 12.5 per cent. + + − − − − − − − − − − − − − −

Total vaccinated = positive 15 or 57.7 per cent. + + + + + + + + + + + + + + + − − − − − − − − − − −

That the administration of vaccine has an effect on the complement fixation reaction of pertussis cases seems indisputable, but it is doubtful if complement fixing substances would develop in the serum as the result of vaccine alone. Among the twelve vaccinated children that did not develop typical whooping cough (Table 4), the serum of one only, who had coughed for two weeks, gave a positive complement fixation reaction with two Bordet-Gengou antigens (a percentage of 8.3) and this was probably a case of whooping cough running a short atypical course. Two normal adults, inoculated with large doses of vaccine (Table 7), at no time gave a positive complement fixation reaction when inactive serum was used. Before the first inoculation a positive reaction was given by the active serum of O., who had had pertussis twenty-one years before, with antigens P. D., 10 and BI_2. The active serum of L., who never had had pertussis, gave a positive reaction after the sixth injection with antigen Z, but several months before this experiment was performed his active serum gave a positive reaction with antigen C.

TABLE 7.

RESULTS OF SUCCESSIVE INCREASING DOSES OF PERTUSSIS VACCINE ON COMPLEMENT FIXATION IN THE CASE OF TWO NORMAL ADULTS.

	Date of Injection	Amount of Vaccine 1 Injected in Millions	Reaction	Date of Bleeding	Date of Test	Results											
						Antigen P. D.		Antigen 98		Antigen C		Antigen 10		Antigen BI$_2$		Antigen Z	
						Active Serum	Inactive Serum	Active Serum	Inactive Serum	Active Serum	Inactive Serum	Active Serum	Inactive Serum	Active Serum	Inactive Serum	Active Serum	Inactive Serum
				4/ 1/14	4/ 7/14	++++	–	....	.....	....	.....	+++	–	+++	–	......	.....
O. (Had pertussis 21 yrs. ago)	4/ 1/14 4/ 4/14 4/ 7/14 4/11/14	125 250 500 1,000	 Local. . . . . Strong local. Local and slight general.	4/ 7/14	4/ 7/14	±	–	....	.....	....	.....	–	–	–	–	......	.....
	4/21/14	2,000	Strong general; headache, dizziness, etc.	4/29/14	5/ 4/14	Strong +	+	....	.....	....	±	....	±	....	.....	Strong +	±
				4/ 1/14	4/ 7/14	Strong +	–	–	–	....	.....	–	–	±	–	......	.....
L. (Never had Pertussis)	4/ 1/14 4/ 4/14	125 250		4/ 7/14	4/ 7/14	–	–	–	–	....	.....	–	–	–	–	......	.....
	4/ 7/14 4/11/14 4/14/14	500 1,000 2,000	Local. Strong local. . . . . . . Slight general; temperature 99.1.	4/14/14	4/20/14	......	+	–	–	....	.....	....	.....	....	.....	......	.....
	4/21/14	4,000	Temperature 100.2; muscular weakness	4/29/14	5/ 4/14	Strong +	+	....	.....	....	±	....	±	....	.....	+++	±

TABLE 8.

Results of Seventy-Seven Comparative Complement Fixation Tests with the Active and Inactive Serum of Thirty Specimens from Cases of Pertussis.

Antigens	Number of Specimens Tested	Number of Specimens Giving Positive Reaction	
		Serum Inactive	Serum Active
P. D.	10	..	2
C.	22	1	4
I.	9	..	..
31	12	..	..
10	1	..	..
87	1	..	..
BI_2	16	..	3
Gonococcus	6	..	5

These tests on the serum of normal adults demonstrate the danger of making false diagnoses by using active serum. In determining the etiology of pertussis, active serum is of little value. We have tested thirty specimens from pertussis cases, active as well as inactive, some with several antigens, so that seventy-seven comparative tests have been made (Table 8). Of ten tests with P. D. two more positives were obtained with active serum. Of twenty-two tests with C. three more positives were obtained with active serum. Of sixteen tests with BI_2, positives were obtained with active serum only, three in number. Five specimens from whooping cough cases with no history of gonococcus infection gave a positive reaction with a specific gonococcus antigen when tested active; the inactivated serum reacted negatively or doubtfully. Most of the specimens of active serum used as negative controls in the pertussis tests gave negative or doubtful reactions with antigens of the Bordet-Gengou bacillus, but even an occasional false positive is sufficient to destroy the diagnostic value of the test. A negative reaction given by active serum is stronger evidence of the lack of a specific infection than is a negative reaction given by inactive serum; but a positive reaction given by active serum is, in our experience, no proof of the presence of a specific infection.

The tests made at room temperature for four hours and at ice-box temperature for four hours have been too few to warrant the drawing of conclusions concerning the value of either method as compared with water bath or incubator fixation; but they are sufficient to show that not all convalescent cases of whooping cough give a positive reaction even when four hours are allowed for fixation. Eighteen specimens (eight vaccinated convalescents, nine unvaccinated convalescents and one vaccinated doubtful case) were tested against Bordet-Gengou antigens by both water bath and room temperature methods and four more positives (22.2 per cent.) were obtained by the latter method. Two of these were among vaccinated convalescents, one was an untreated convalescent and the fourth was a prophylactic case. Five vaccinated convalescents gave positive reactions by both methods; one vaccinated convalescent was negative by both; eight untreated convalescents were negative or doubtful in both. Other tests were made that could not be read at all, owing to the anticomplementary action of the antigens. When the temperature of the room is so variable that a standardization of the antigen is no guide to the amount of antigen to be used in the test, this method is not of practical value. We have not tested enough negative controls by the room temperature method to be certain of the specificity of the reaction. Experience in the diagnosis of other infectious diseases by means of complement fixation led us to suppose that constant and reliable results would be obtained by ice-box fixation and a higher percentage of positives than by water bath or incubator fixation. The number of tests made is insufficient to establish this. Thirteen specimens have been tested against Bordet-Gengou antigens, allowing four hours for fixation. Only two more positives (15.4 per cent.) were obtained by this method than by water bath fixation. One was in the case of a vaccinated convalescent, the other an untreated convalescent. One specimen gave a positive reaction by both methods. Of the nine specimens that gave a negative or doubtful reaction by both methods one was from a vaccinated whooping case, one from a vaccinated convalescent and the other seven from untreated cases still whooping. A longer period than four hours would probably give equally specific results and more positive reactions.

As our technic improved the percentage of positive reactions given by the serum of pertussis cases has increased, but even among pertussis convalescents the number of positive reactions is still far below 100 per cent. Even though the power of fixing complement with antigen of the Bordet-Gengou bacillus is not constantly present in the serum of these cases, the frequency of a positive reaction is so great as to be presumptive evidence of the etiological relationship of the Bordet-Gengou bacillus to whooping cough.

SUMMARY

1. The most reliable antigen for complement fixation tests in whooping cough is obtained by autolyzing an aqueous emulsion of a twenty-four to forty-eight hour growth of the Bordet-Gengou bacillus for eighteen to twenty-four hours at 56 C. and shaking for several hours. The closeness of relationship of the Bordet-Gengou strains is still under investigation. To obtain the maximum number of positive reactions it may be necessary to use a polyvalent antigen.

2. Active serum may give non-specific positive reactions. A negative reaction given by active serum is stronger evidence of the lack of an infection than is a negative reaction given by inactive serum.

3. For fixation one-half hour in the water bath or one hour in the incubator is absolutely reliable. Ice-box fixation probably gives reliable results; the optimum period is still to be determined. Fixation at room temperature for four hours is unsatisfactory, at least in rooms with variable temperature.

4. About 40 per cent. of whooping cough cases have given a positive reaction with antigens of the Bordet-Gengou bacillus, when inactive serum was used. The highest percentage of positives is given by convalescent vaccinated cases. Ten per cent. of whooping cough cases have given a positive reaction with antigens of hemoglobinophilic strains.

5. A ++, +++, or ++++ reaction by inactive serum with an antigen of the Bordet-Gengou bacillus is diagnostic of whooping cough, a + or ± reaction is suspicious, a negative reaction has little significance.

CONCLUSIONS

Complement fixation tests on serum from one hundred eleven cases of pertussis or suspected pertussis support the theory that the Bordet-Gengou bacillus is the etiological factor in the disease. The complement fixation test may be of value in the diagnosis of doubtful cases of pertussis.

STUDIES ON THE CULTIVATION OF THE RABIES VIRUS

Caroline R. Gurley and Charlotte C. Van Winkle

Introduction

The work reported below was pursued under the direction of Dr. A. W. Williams, Assistant Director of the Research Laboratory, from whom helpful suggestions and criticism were received.

In September, 1913, Noguchi[1] first published upon the cultivation of the rabic virus, by a method similar to the one with which he had successfully cultivated the spirochaete of relapsing fever.[2]

In cultures from "street," "passage" and "fixed" virus brains, including many generations of transplants, he found very minute granular and somewhat coarser pleomorphic chromatoid bodies. The microphotographs do not bring out the character of these granules definitely. Also in cultures from four cases, including "passage" and "fixed" virus, in which the granules mentioned were present, he found totally different bodies, nucleated, round or oval, surrounded with a membrane. These suddenly appeared, lasted four or five days, and diminished, coincidently with an increase in the granular bodies. The nucleated bodies appeared to him to be actively multiplying by division or budding, and to be protozoa.

Cultures containing the granular, pleomorphic, or nucleated bodies, produced rabies in dogs, rabbits, and guinea pigs, as shown by typical symptoms and positive animal inoculations. Smears from the brains showed always the granular, and sometimes the nucleated bodies in large numbers.

With regard to transmission, Williams[3] raised the question as to the number of culture generations through which some of the original rabies virus unchanged by growth might be transferred.

By the use of dog's serum and later of Ringer's solution, Moon[4] attempted to cultivate the virus of rabies. He produced rabies in one guinea-pig with the fifth and in one with the sixth subculture. Controls in normal salt under oil produced rabies in the second subculture only.

Previous to Noguchi's paper mentioned above Proescher[5] had demonstrated by a special stain an antiformin-resistant organism with coccus, bacillar and spirochaete forms, in street virus dogs' and in fixed virus rabbits' brains. The same organism he found in the rabic salivary glands of three dogs and in the salivary glands and nerve ganglion of a cow. He interpreted the different forms, and considered that "the exclusive staining properties of the rabic virus and its almost achromatophilia to all aniline dyes except azur carbonate almost establish the fact that this is a specific microorganism."

Later[6] the same author reported the production of rabies in rabbits, rats and a monkey with cultures of this organism, including the eighth subculture. He believes the etiology of rabies established.

Whether the coccoid bodies described in 1910 by Joseph Koch[7] were identical with the organism of Proescher remains unsettled.

Volpino[8] in repeating Noguchi's work, found polymorphic forms completely resembling those described by Noguchi, but later observed them in ascitic tubes without rabic brain. He considered the bodies lipoid drops.

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[1] Journ. Exper. Med., 1913, xviii. No. 3, 314-316; 1 pl.
[2] Journ. Exper. Med., 1912. xvi. 199.
[3] Journ. Am. Med. Assn., 1913. lxi. 1509.
[4] Journ. Inf. Dis., 1913. xiii, 232-235.
[5] N. Y. Med. Journ., Jan. 4. 1913.
[6] N. Y. Med. Journ., Nov. 14. 1914.
[7] Deutsche med. Wchnschr., 1913, xxxix, No. 42. 2025-2029.
[8] Presse med., 1914. No. 8, 79.

Levaditi[9] reported the cultivation of the rabic virus, as previously that of the poliomyelitic virus, in symbiosis with the cells of the spinal ganglia of the ape, incubated at 37°C., in the plasma of this animal, under albolene. The fifth generation—each generation representing 6 days of culture—produced rabies in rabbits, after 10 to 11 days. He does not touch upon morphological findings in his cultures.

Investigation

The work here reported on, was undertaken to determine with what regularity the cultivation of rabic virus by the method reported by Noguchi[2] could be obtained.

The fresh brains of "street" and "fixed" virus animals were used. The former represented either the first, second or third passage in the guinea-pig, of virus from the dog and, in one instance, the cat; the latter were derived from rabies of passages from 818 to 828, with two exceptions, namely, 710 and 711.

Starting of Cultures—The street virus guinea-pigs were allowed to die, or were chloroformed, after the development of unmistakable symptoms. The brain was removed under asceptic conditions, and cut into pieces equivalent to cubes of about 5 mm. in diameter.

A serious difficulty was encountered in obtaining uncontaminated rabic brains, as in a number of cases, the brain control—that is, the ascitic fluid tube with no kidney but a block of the brain—showed bacterial growth. This occurred with both street and fixed virus brains, and led, in the case of some of the street-virus animals, all of which were inoculated especially to furnish culture material, to chloroforming after the onset of typical symptoms. The hope was to eliminate terminal secondary infections.

Preparation of Medium—The kidneys were obtained by chloroforming a normal rabbit, the blood being collected for culture media. Immediately after bleeding, the animal was turned over and the back wet with 5 per cent. carbolic solution, which was thoroughly rubbed in with absorbent cotton. Twenty to thirty minutes later, the subcutaneous tissues were laid bare, carbolic solution poured over, and with fresh instruments, incisions were made and the kidneys removed. These were cut into about eight blocks, each being transferred to a tube of ascitic fluid somewhat more than half full. One tube representing each kidney, was incubated for about 48 hours at 37°C., and a smear examined. If this control proved sterile, the others were assumed to be so. In the meantime and until wanted, the other tubes were kept in the ice chest. None older than 5 days, and usually none older than 3, were used. The ascitic fluid was always raw, never artificially sterilized. During the early part of the work, we experienced considerable difficulty from contaminations of individual tubes, and, to a smaller extent, this continued.

Inoculation of the tubes with the brain was done just after its removal, the piece sinking until it rested on the block of kidney. A layer of sterile albolene, three-fourths to an inch deep, was added, and the tubes divided into a set for 37°C. and one for room temperature. To allow for discarding of contaminated tubes, and to have extra ones for making smears before transplanting time, more tubes were inoculated than were otherwise needed. As controls, a tube of ascitic fluid with the rabic brain and one of ascitic with kidney, were used.

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[9] Compt. rend. Soc. Biol., 1913, lxxv, No. 35, 505.

Transplants—These were made from incubator cultures, once in 7 to 9 days; those of room cultures, once in 12 to 14 days. A certain amount of work was done in the way of varying the interval, but no advantage accrued.

About 5 c.c. of the culture fluid, taken from around the brain and kidney, was used for transplanting. It was carried over, care being taken to disturb the contents as little as possible, by means of a pipette drawn at one end into a capillary tube and provided with a rubber bulb. When transplanting, smears were made, and previously a smear from one or more tubes had been examined every other day, and in many cases oftener. Smears were usually stained with Giemsa, sometimes with the stain employed for Negri bodies.[10]

Animal Inoculations—Subdural inoculations into two guinea-pigs were done, with the exceptions, in some cases, of first generations, and second generations in the case of room cultures, it being a foregone conclusion that these would transmit.

Special precautions were found necessary in inoculating, in order to rule out chance of infection from the wound. The hair over the head was closely clipped, cleansed with alcohol, a small incision made, and a hole drilled for the needle. After the injection, tincture of iodin was applied with a camel's hair brush, aristol sprinkled on freely, and thick celloidin solution dropped on, to make a firm adhesive dressing.

At autopsy, smears, and cultures on blood-agar plates, were taken from the heart's blood and from any focus of infection. Smears from the brain were stained for Negri bodies.

In determining infectivity several difficulties arise: As has been noted by different authors (a) animals with rabies do not always show definite symptoms; (b) definite Negri bodies are not always found; and (c) in very rare cases an animal fails to succumb.

Our experience afforded instances of each of these contingencies, especially of lack of characteristic symptoms. Failure to succumb occurred with 5 guinea-pigs inoculated subdurally with different strains of street virus kept in glycerin for periods of 33 to 104 days (see table).

Case	No. of Days in Glycerin	Time Watched	Case	No. of Days in Glycerin	Time Watched
A	81 days	13 weeks	D	36 days	?*
B	33 days	13 weeks	E	104 days	7 weeks
C	55 days	6½ weeks	F	65 days	Died after 7 weeks

*Discharged long after the upper time limit; probably observed several months.

Prolonged incubation with street virus—7 weeks—occurred in one guinea-pig (F in table), inoculated with material in glycerin 65 days; and in another—46 days—inoculated with an early culture generation.

Another case of prolonged incubation with atypical course, was afforded by a guinea-pig inoculated with a street virus dog's brain in glycerin 16 days. Of 2 inoculated, one guinea-pig died at the end of 36 days after typical symptoms; the other showed questionable beginning excitement on the 13th day, typical symptoms on the 14th, and an apparent return to normal on the 15th. Further symptoms were not noticed, and death occurred over a Sunday, that is, on the 23d or 24th day. Another aberrant case was that of a guinea-pig inoculated with street virus in glycerin 53 days, which showed symptoms on the 14th day, was apparently well on the 15th, but had a return on the 16th. Death did not occur until the 7th day after the first definite symptoms.

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[10] Pathogenic Micro-organisms. Park and Williams, 5th Ed., New York, 1914, p. 83.

Culture Work

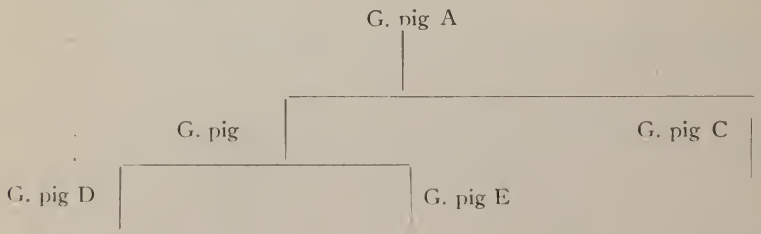
The data here given, mainly rest on 15 series of street virus cultures and 9 of fixed virus. A comparatively large number of other series started, are not included, as, in the majority of them, the brain was found contaminated. Among these were four street-virus brains (3 dogs', 1 human), which were sent to the laboratory without sterile precautions. Apart from these, however, especially early in the work, a considerable number of the brains of animals inoculated expressly to obtain virus free from contamination, showed bacterial growth in the cultures. The proportion of such was later reduced by the technic in inoculating described above, and in some cases by chloroforming before death.

Chromatoid granules varying from just visible specks to those more or less resembling very small cocci (often including indefinite forms) were found in certain culture and control tubes, the latter being ascitic fluid containing either kidney or normal brain, whichever was selected for culturing the rabbit brain in question. The rather more definite type of granules occurred in tubes (whether cultural or control) containing brain instead of kidney. Nevertheless, cultures which proved virulent might show no granules at all.

The nucleated forms resembling Negri bodies, found by Noguchi in four of his cases, were seen by us in only two or three smears out of a very large number examined, were even then very rare, and were not as definite as his. Similar bodies were once seen in a control tube of ascitic fluid with kidney. It is possible that with Heidenhain's iron haematoxylin method, recommended by Kraus and Barbara to demonstrate them, more might have been seen.

In cultures from the brains of guinea-pigs inoculated with street virus from 3 dogs and a human case, yeasts were found and isolated on glucose-agar plates. Also from the brain of a fixed-virus rabbit (passage 818), yeasts were isolated, and in one tube only, from another (passage 823), probable yeasts were seen. No definite yeasts were demonstrated in smears from guinea-pig's brains which in culture yielded them.

The most striking series of yeast infections accompanying rabies arose from one of the dogs mentioned. Guinea-pigs were inoculated as follows:



From guinea-pig A no cultures were made; in cultures from guinea-pigs, B, C, D, E, yeasts were present and isolated. The colonies on blood-streaked agar plates were pigmented (orange); no mycelium was produced on plates or in liquid cultures. The yeasts from the fixed virus rabbit also showed no mycelium; their colonies were white.

From the case of the guinea-pigs above, it is clear that the yeasts survived during the life of the animal, and were transmitted along with the rabic virus. The course of the disease appeared to be unaffected by the mixed infection.

Liquid cultures containing yeast showed, besides unmistakable ones usually surrounded by broad, transparent capsules, many forms unrecognizable as yeasts but which probably were such. This pleomorphism was perhaps due to the semi-anaerobic condition, and was for a time puzzling.

Duration of Virulence—While too few experiments were done to set limits to variation with different strains, case 1 (see table) furnished the suggestion that, as others have shown, the virus dies out sooner at incubator than at room temperature. Thus at room temperature, generation 1 produced rabies after 23 days' cultivation, and the corresponding incubator tube did not. Also generation 2 at room temperature, brought down from a 25-day old culture, and the incubator culture did not.

Room Cultures				Incubator Cultures			
Case No.	Generation No.	Age of of Culture	Rabies Produced	Case No.	Generation No.	Age of Culture	Rabies Produced
1	1	32 days	In 1 g. pig and probably in the second	1	1	32 days	None in either guinea pig
1	2	25 days	In only g. pig inoc.	1	2	25 days	None; only 1 g. pig inoc.
2	1	46 days	In only g. pig inoc.	4[1]	1	33 days	None in either guinea pig
3	1	23 days	In only g. pig inoc.	4[2]	1	35 days	None in either guinea pig
				5	1	21 days	None; only 1 g. pig inoc.

[1] Fixed virus culture kept in anaerobic jar.
[2] Fixed virus culture kept under albolene but not in anaerobic jar.

Both street and fixed virus room cultures, in generations 1 and 2, showed a considerably higher infectiousness than the corresponding incubator cultures.

Street virus in generation 3, incubator, showed a higher rate of transmission than in 1 or 2, but as this was reduced almost to zero in generations 4 and 5, the rise cannot have been significant.

Fixed virus, generations 1 and 2, at both temperatures, seemed to transmit better than street, as would be expected; in higher generations, cases of certain transmission were too few to warrant any comparison. With fixed virus, generation 1 at 37°C. and generation 2 at room temperature, transmitted to about 50 per cent., but the falling off in generation 3 was down to 12 or 14 per cent., and in higher generations was almost negligible, when the following anomaly is disregarded.

Generation 5 of cultures at 37°C. of one fixed virus rabbit (passage 822) produced death in guinea-pigs as below:

Guinea-pig 1 died in 9 days; very suspicious Negri bodies in brain.
Guinea-pig 2 died in 12 days; suspicious Negri bodies in brain.
Guinea-pig 3 died in 16 days; suspicious Negri bodies in brain.
Guinea-pig 4 died in 3-5 days; *slightly* suspicious Negri bodies in brain.

The brains of numbers 1, 2 and 3 failed to transmit; that of number 4 was not inoculated. In guinea pigs 1 and 3, autopsy showed quite extensive consolidation of the lungs; in 2 and 4 no sufficient cause for death was found.

At the suggestion of Dr. Williams, in 4 cases attempts were made to cultivate fixed virus with normal brain substituted for the kidney. This procedure gave transmission results certainly as good. In neither case, however, was multiplication established.

Where cultures produced rabies, we found a lengthening of the incubation period roughly corresponding with the culture generation inoculated.

CONCLUSIONS

1. No evidence was found of multiplication of the rabic virus.

2. The Negri-like bodies found by Noguchi in four cases, were, in our experience, very rare, and were not definite. What may have been the same were once seen in a control tube of ascitic fluid with kidney.

A TEST FOR ANTITHROMBIN IN THE BLOOD*

Alfred F. Hess

(*Reprinted from the Journal of Experimental Medicine, vol. xxi, No.* 4, 1915.)

The part that antithrombin plays in the human blood in inhibiting coagulation is a question concerning which there is a decided difference of opinion. Although it is admitted generally by physiologists that antithrombin is present in the circulating blood, there is a marked divergence as to the significance and importance which should be accorded it. On the one hand, we note that Morawitz, whose theory of coagulation has gained wide acceptance, assigns to antithrombin no function in the theory of coagulation which he has elaborated. This omission seems to be a weak link in the chain which he has constructed, as any theory is necessarily incomplete which leaves out of consideration a substance which is regularly present in the circulating blood. On the other hand, Howell considers antithrombin to be a very important constituent, ascribing to it the part of maintaining the fluidity of the blood, in that coagulation ensues only when the antithrombin is rendered inert by the neutralizing effect of the zymoplastic substance in the shed blood or in the tissues. As stated, however, there is no diversity of opinion regarding the normal occurrence of antithrombin, so that it would seem worth while to study this substance clinically from a quantitative point of view, in order to obtain fuller data from which to judge its importance. The term antithrombin is employed in a functional sense to designate any substance or substances in the plasma which tend to inhibit coagulation. It is realized that a terminology of this kind cannot be absolutely satisfactory, especially from a chemical point of view. Nevertheless, it appears to be justifiable; it is, for example, in accordance with the physiological studies in immunity, where all the substances—complement, amboceptor, etc.—exist only from a functional viewpoint.

There have been very few quantitative examinations of antithrombin in the human blood. This, probably, may be accounted for by the fact that it has been overshadowed by the striking importance of other substances in the blood—the thrombin, fibrinogen, and other active principles, which may be grouped as the positive coagulative factors—and in part by the fact that there has been no simple method for making such tests. The few estimations of this nature which have been carried out have been reported by Howell, who used a method which he has devised. Although this method seems to be satisfactory, it is by no means simple, and is hardly suited to wide clinical use. In the first place, it requires the preparation of a pure thrombin. This is extracted from pig fibrin by treating it with an 8 per cent. solution of sodium chloride, followed by repeated extractions of the coagulable proteins by means of chloroform. The test also requires the preparation of a solution of fibrinogen which must contain no prothrombin; that is, must not clot merely upon the addition of calcium. It requires considerable experience to prepare both of these substances satisfactorily; the fibrinogen solution, which is not the salted fibrinogen of Hammarsten, but a dialyzed plasma, is especially difficult to obtain and to maintain free from contamination of prothrombin.

In the course of testing the coagulability of oxalated plasma from various sources, an examination which included a test for antithrombin, it was found that for clinical use a simple method could be employed, which requires neither a preparation of thrombin nor of fibrinogen. The method is carried out as follows:

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* Presented before the Society for Experimental Biology and Medicine, November 18, 1914.

About nine cubic centimeters of blood are aspired and put into one cubic centimeter of 1 per cent. sodium oxalate. The blood is centrifugalized and the plasma removed in the usual way. The plasma is then recalcified by adding 2, 3, 4 and 5 drops, respectively, of a 0.5 per cent. calcium chloride solution. In this way we ascertain the general coagulability of the plasma, which is the composite of a number of interacting factors—prothrombin, fibrinogen, antithrombin, etc.—and we determine at the same time the optimal amount of calcium for this particular plasma.

If we heat some of this plasma to 60° C., the prothrombin, as is well known, is destroyed and the fibrinogen is coagulated. After filtering off the coagulum we have a plasma which contains antithrombin and practically no prothrombin. The strength of this antithrombin may be ascertained, for clinical purposes, as follows:

First, we prepare human plasma from a normal case just as we prepared the oxalated plasma which is to be tested. Five drops of this plasma are put into five thoroughly cleansed vials. The first of these serves as a control; to the second three drops of normal antithrombin are added; to the third five drops of normal antithrombin; to the fourth three drops of antithrombin that is to be tested, and to the fifth five drops of this antithrombin. All tubes are equalized in amount by the addition of normal salt solution, and the mixtures are allowed to remain in contact for fifteen minutes. The plasma is then recalcified by the addition of 0.5 per cent. calcium chloride, the number of drops which are added having been determined by the general coagulability test, which should always precede the antithrombin test. As a rule, four drops have been found to be the optimal amount.

TABLE I.

ANTITHROMBIN TEST.

A.

Normal			Hemophilia		Interval
Control	3 Drops Antithrombin	5 Drops Antithrombin	3 Drops Antithrombin	5 Drops Antithrombin	
–	–	–	–	–	2 min.
+	+	+	+	+	4 min.
+++	+++	++	+++	++	6 min.
		+++		+++	8 min.

B.

Normal			Purpura		Interval
Control	3 Drops Antithrombin	5 Drops Antithrombin	3 Drops Antithrombin	5 Drops Antithrombin	
–	–	–	–	–	4 min.
+	+	+	+	+	6 min.
+	+	+	+	+	8 min.
+++	++	++	+	+	10 min.
	+++	++	++	++	12 min.
		++	+++	++	14 min.
		+++		+++	16 min.

Table I *A* illustrates a test of this kind in an atypical case of hemophilia where there was a deficiency of calcium in the blood. We note that antithrombin was present in the plasma of the patient to no greater degree than in the normal plasma; in both instances coagulation was rapid and but slightly delayed by the addition of three and of five drops of antithrombin. Table I *B* illustrates a similar test in a case of purpura. Here we likewise find no increase in antithrombin.

Antithrombin is judged to be in excess where a marked delay in coagulation is brought about in the tubes to which it has been added, as compared with the coagulation in the control tube. As in the case of the coagulation time, no arbitrary norm can be set up; the results should be well defined to warrant the conclusion that there is an excess of antithrombin.

The essential difference between this antithrombin test and that of Howell is that plasma is used as a basis instead of a fibrinogen solution. The validity of employing plasma in this way may be determined by preparing solutions of hirudin of varying strengths and titrating them upon plasma; in other words, by substituting hirudin for human antithrombin. Tests of this nature were carried out, first upon horse plasma, and later upon normal human plasma. In both cases the plasma was clear and had a coagulation time of not over ten minutes. Table II *A* illustrates an experiment of this kind. We may note that dilutions of 1 to 20,000, 30,000 and 40,000 of hirudin were employed, and that in each instance 1, 3 and 5 drops of one of these antithrombin solutions were added to the human plasma. It will be seen that in each of the three tests the sequence of coagulation is in direct ratio to the number of drops of antithrombin added, and that in general the more dilute the hirudin, the less its inhibiting effect. A test (table II *B*) of normal human antithrombin upon its own plasma has been added to this table to enable a comparison between the strengths of the hirudin used and of human antithrombin. It will be seen that, according to the test, human antithrombin is about equal to a 1 to 40,000 solution of hirudin.

If we turn again to table I, we notice that in addition to the tests of plasma in the case of purpura and of hemophilia, it includes titrations of antithrombin upon autogenous plasma. In these instances the plasma was normal. The same autogenous test may be carried out in pathological cases, *e. g.*, in hemophilia, as shown in table III, which reproduces an equilibrium test of this kind with the same plasma which is reported in table I. This test evidently cannot be considered an antithrombin test, as we employ plasma from a pathological case in order to ascertain the degree of antithrombin. It is termed an equilibrium test because it gives us information as to the balance which obtains in the plasma between all positive and negative factors concerned in coagulation. If the balance is in a state of delicate adjustment, the addition of a small amount of antithrombin will suffice greatly to delay coagulation, whereas if there is an excess of prothrombin and allied substances, this addition will bring about but a slight increase in the coagulation time. We see when we compare the two tables. In table I, where two equilibrium tests of normal plasma are shown, the addition of three drops of antithrombin resulted in either slight or no delay, and five drops brought about at most a retardation of from ten to sixteen minutes. These tests must not be considered exceptions to the rule, although the delay when three drops of antithrombin are added is generally more marked, and when five drops are added, the period of complete coagulation is postponed to fifteen or twenty minutes. On the other hand, with the plasma of the hemophiliac, referred to in table I, the addition of the same amounts of antithrombin was sufficient to retard coagulation markedly (table III). Comparative tests such as these demonstrate that the mere coagulation time does not furnish complete information as regards the power for clotting which exists in plasma. There are latent potential coagulative factors which come to light only when inhibiting substances are added to the plasma. For example, a normal plasma (table II *A*) coagulated in ten minutes; the plasma of the hemophiliac (table III) in twelve minutes, that is, almost in the same length of time; nevertheless, upon the addition of five drops of its own antithrombin, the former coagulated in sixteen minutes, whereas the latter took forty-three minutes to clot. This test is not recommended for clinical use, because the testing of antithrombin upon abnormal plasma is erroneous. However, it is highly significant from one point of view: it shows that the circulating blood is not delicately balanced in regard to its coagulability. When we reflect that in adding three and five drops of antithrombin to five drops of plasma we are more than doubling its normal content of antithrombin, and that nevertheless coagulation generally ensues with but slight retardation, we must conclude that there exists a considerable factor of safety in the mechanism of the coagulation of the circulating blood. If such were not the case, serious hemorrhage, as the result of a slight temporary excess of antithrombin, would be a constant danger.

TABLE II.

A. Hirudin.

Dilution 1 : 20,000

1 Drop Hirudin	3 Drops Hirudin	5 Drops Hirudin	Interval
−	−	−	5 min.
+	+	−	8 min.
++	+	+	10 min.
++	++	+	11 min.
++	++	+	12 min.
+++	++	++	14 min.
	++	++	16 min.
	+++	++	18 min.
		++	20 min.
		+++	22 min.

Dilution 1 : 30,000

1 Drop Hirudin	3 Drops Hirudin	5 Drops Hirudin	Interval
−	−	−	5 min.
+	+	+	8 min.
++	++	+	10 min.
+++	++	++	11 min.
	++	++	12 min.
	+++	++	14 min.
		+++	16 min.

Dilution 1 : 40,000

1 Drop Hirudin	3 Drops Hirudin	5 Drops Hirudin	Interval
–	–	–	5 min.
+	+	+	8 min.
+++	++	++	10 min.
	++	++	11 min.
	++	++	12 min.
	+++	++	14 min.
		+++	16 min.

B. ANTITHROMBIN TEST.

Control	1 Drop Antithrombin	3 Drops Antithrombin	5 Drops Antithrombin	Interval
+	+	+	–	5 min.
++	++	++	+	8 min.
+++	+++	+++	++	10 min.
			++	11 min.
			++	12 min.
			+++	14 min.

TABLE III.

EQUILIBRIUM TEST.[1]

Control	3 Drops Antithrombin	5 Drops Antithrombin	Interval
–	–	–	6 min.
+	+	+	10 min.
+++	+	+	12 min.
	+++	+	15 min.
		++	35 min
		+++.	43 min.

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[1] The same case as in table I *A*.

SUMMARY

A test is described for the estimation of antithrombin in the blood. The chief advantage of the test is that it is simple, and does not require the preparation of fibrinogen and of thrombin, which are difficult to prepare and to maintain in a pure state. The principle consists in titrating the antithrombin against normal human plasma; in this way we obtain an estimation of its power to delay coagulation. As the result of examinations carried out by this method, it would seem that there is a wide factor of safety as regards the amount of antithrombin in the human blood, and that this inhibiting substance may be increased to a considerable degree without markedly delaying or endangering clotting.

THE GASTRIC SECRETION OF INFANTS AT BIRTH*

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There has been no lack of investigation of the gastric secretion of infants. Shortly after the stomach tube was devised by Kussmaul it was carried into the field of pediatrics, first for therapeutic measures and considerably later for physiologic studies. In spite of many years of varied work in this field, it must be admitted that we have not progressed far in our knowledge of the physiology of gastric digestion. Numerous causes may be assigned for this slow progress. One of the main obstacles to a scientific study of secretion—the phenomenon which interests us particularly at this time—has been and remains the fact that the acid juice rapidly enters into combination with the milk food, so that it becomes impossible to judge accurately of its quantity or of its quality. It is this variable factor which accounts for the wide discrepancies in the reports on this subject, for the differences of opinion regarding the importance of hydrochloric acid at this time of life, and for the total lack of normal values for the gastric secretion of infants.

The present study, which was carried out in conjunction with an investigation of icterus neonatorum and its relation to bile secretion,[1] *concerns itself merely with infants during the first few hours of life, who have never been given any food.* Thus the disturbing and complicating factor of the interaction of the gastric juice and of the food does not have to be considered. Quite apart from the question of whether a study from this limited point of view might prove of clinical value, it seemed worthy of investigation for its physiologic interest, because, as we have said, from its very nature it eliminated the food factor and reduced a complex subject to its simplest terms. The questions of suitability of diet, of previous gastric derangement, or of intercurrent illness, in fact, all complicating factors, excepting those possibly congenital in origin, were also naturally excluded.

Our study includes a gastric examination of fifty-five infants, varying in age from one-half hour to eighteen hours. Such a considerable number of tests must not be regarded as indicating a lack of uniformity in the results. They were carried out to this extent because, as has been mentioned, this work was performed in conjunction with another experimental study—that of the duodenal contents of the new-born. Indeed, attention was first directed to this question in the course of investigations by means of the duodenal catheter.

A review of the literature of the gastric secretion of infants revealed practically nothing on this phase of the subject. The only published account of examinations of the contents of the stomach of new-born (unfed) infants are two by Leo,[2] and a solitary test made by Szydlowski[3] many years ago. Some post mortem reports were found: Zweifel,[4] in 1874, demonstrated the presence of pepsin in the gastric mucosa of the new-born, and Langendorff,[5] a few years later, found this ferment in the mucous membrane of a four-months-old fetus.

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* Presented in abstract before the Society for Experimental Medicine and Biology, February, 1912.

[1] Hess, A. F.: Am. Jour. Dis. Child., 1912, iii, 304.

[2] Leo. H.: Berl. klin. Wehnschr., 1887, xxiv.

[3] Szydlowski, Z.: Jahrb. f. Kinderh., 1892, xxxiv, 411.

[4] Zweifel, O.: Untersuch. ueber d. Verdauungsapparat d. Neugeb., 1874.

[5] Langendorff, O.: Arch. f. Anat. u. Physiol., lxxix, 95.

As the subject seemed worthy of further and closer investigation, it was entered on in detail to determine the presence of free hydrochloric acid, of pepsin, of rennin, of gastric lipase; the degree of secretion and its duration; its relation to the general physical condition of the infant; the relation of the hydrochloric acid secretion to tonicity of the pylorus; its relation to the pancreatic secretions; its relation to later gastric secretion, at a period when the babies had been fed. Finally an attempt was made to interpret the nature of the secretory stimulus in the light of our own tests and of present physiologic viewpoints.

We have summarized the results in tabular form (Table 1). A glance at this table shows that in almost every test hydrochloric acid was found; it was lacking in only one out of 52, and was found in the free state in all but two. The infants varied in age from one-half hour to eighteen hours, and in no instance had received any food or water. The method of obtaining the gastric contents differed from that usually employed only in the use of a glass aspirating bulb.[6] There is no doubt that we did not, at all times, completely empty the stomach. Bearing this in mind, it is the more interesting to find that these new-born infants showed as much as 8 to 10 c. c. of highly acid gastric juice, containing a large amount of free hydrochloric acid. The juice was clear or slightly turbid, somewhat viscid, due to an admixture of mucus, which secreted freely in this early period, and contained a varying amount of saliva. The saliva is not included in the figures giving the total quantity; as far as possible it was removed from the surface of the fluid, so as not to interfere with the acidity tests. For the test of hydrochloric acid Congo paper was used in the clinic, and Toepfer's solution as an indicator for the titration in the laboratory; for the test of combined hydrochloric acid alizarin was employed. We do not wish to insist on the quantitative accuracy of these tests.[7] However, they are probably not too high. For from a quantitative standpoint, all intercurrent disturbing factors, such as admixture of mucus or of saliva, tend to lower the figures for free hydrochloric acid from their level rather than to raise them. Moreover, the figures would seem more accurate than those obtained from older babies on account of the absence of peptones and alubumoses which interfere with the activity of the test indicators. We may therefore state not only *that very soon after birth there is free hydrochloric acid, but that there is a considerable amount of this acid in the unfed infant's stomach.* Rennin and pepsin are also present at this time; as a test for the former, milk was used, and for the latter, coagulated egg-white. No quantitative estimation of these two ferments was attempted; their presence in the gastric juice was confirmed several times, and their enzymatic action definite and conclusive. As regards the source and the nature of the lipase, noted in the table, there may be some question. We believe that it is of gastric and not of pancreatic origin, because it was found in fluid which contained no trypsin;[8] Sedgwick[9] found it in the stomach

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[6] It is unnecessary to describe this simple instrument, as it has been reproduced in recent articles in connection with an account of duodenal catheterization (Am. Jour. Dis. Child., 1912, iii, 133: Arch. Int. Med., 1912, x 37.) It has been found exceedingly serviceable in emptying the stomach, for although even with this aid the gastric contents cannot always be completely avacuated, it enables us to obtain a far larger quantity than when aspiration is not employed.

[7] In some instances but 1 or two c.c. of fluid was available for titration, so that error undoubtedly resulted in the course of the necessary multiplication of the figures. However, the fact that in several instances a repeated test showed a repetition of the high acidity argues for approximate exactness. This fact is well brought out by the figures in the case of prolonged gastric secretion which are cited below in full. Moreover it will be noticed that, in general and with slight exception, the amount of hydrochloric acid was found to be excessively high.

[8] For the lipase tests, neutral ethyl butyrate was used as in the studies of pancreatic ferments (Hess, A. F.: Am. Jour. Dis. Child., 1912, iii, 205.) The figures in Table I denote titration against 1/20/n NaOH.

[9] Sedgwick, J. P.: Jahrb. f. Kinderh., 1906, lxiv, 194.

TABLE 1.

GASTRIC SECRETION IN THE UNFED NEW-BORN.

Case	Hours Old	Gastric Juice, c.c.	Free HCl	Comb. HCl	Remarks
1	½	2.0	++	....	
2	4½	10.0	+++	....	
3	4½	8.0	60	....	Pepsin rennin.
4	4	8.0	+++	....	
5	5	3.0	+++	....	14 c.c. in one hour.
6	2	5.0	+++	....	17 c.c. in 1 5/6 hours. (See text.)
7	1⅓	3.0	+++	....	
8	¾	?	+++	....	
9	10	1.5	22	....	
10	6	1.0	15	....	
11	2⅔	8.0	20	32.0	Lipase 0.4
12	5	2.0	+++	....	
13	5	6.0	19	37	
14	17½	1.0	+++	....	
15	12¾	2.0	+++	....	
16	5½	2.0	17.5	17.5	
17	7	2.8	45	36	
21	2½	2.0	+++	....	
22	2	1.0	+++	....	
23	8	2.0	+++	....	
24	5⅓	3.0	+++	....	
25	7½	1.0	+++	....	
26	12	1.0	+++	....	
27	12	1.0	+++	....	Lipase 0.3.
28	18	2.0	+++	....	
29	4	3.0	35	50	
30	1½	4.0	+++	....	Lipase 0.2 rennin.
31	9	2.0	+++	....	
32	14	5.0	+++	....	
33	5¾	0.5	++	....	
34	5½	0.5	0	....	Saliva marked.
35	5	0.25	++	....	
36	5½	0.5	+++	....	
37	15	0.5	++	....	
38	12	1.0	+++	....	
39	5	3.0	+++	....	
40	6	5.0	+++	....	
41	3	2.0	++	....	
42	7	0.3	++	....	
43	3	8.0	+++	....	
44	5½	12.0	+++	....	Rennin pepsin.
45	5½	0.3	++	....	
46	1½	1.5	++	....	
47	3	1.0	++	....	
48	10	?	+++	....	
49	4	5.0	120	....	
50	2½	1.5	+++	....	
51	2½	2.5	+++	....	
52	7¼	5.0	35.0	37.5	Pepsin rennin.
53	....	1.0	++	....	
54	10	?	0	+	
55	9	2.0	+++	....	

of an infant at the age of two weeks, and Ibrahim[10] in a six-months-old fetus.

The secretion of the gastric juice and the elaboration of the ferments were not brought about by the passage of the stomach-tube. This was evident from the fact that the juice was obtained immediately on passing the tube. In order to test the contrary hypothesis, in several cases the catheter was inserted with the greatest dispatch. This did not lessen the amount of gastric juice obtained. According to Pawlow,[11] the gastric mucosa does not react to mechanical stimulation. Moreover, whatever the nature of the stimulus, all physiologists are agreed that a latent period must elapse before the glands respond. As the result of experiments on animals, Cohnheim[12] places this latent period of the gastric mucosa, following psychic stimuli, at about seven minutes. Pawlow[11] gives the same latent period for the reaction to food. In view of experimental as well as of clinical evidence, it therefore seems certain that in our cases the gastric juice was in the stomach before the passage of the catheter, and was not the result of mechanical stimulation. We shall consider later in greater detail the nature of the secretory stimulus after noting some clinical experiments.

Having assured ourselves that the secretion of hydrochloric acid in new-born infants is the rule, almost without exception, we undertook a more precise investigation of the question. One of the first subjects of inquiry was as to the continuance of the secretion—whether hydrochloric acid and the ferments are in the stomach at the beginning of the test only, or whether they can be obtained throughout long periods. Many tests were made to elucidate this question. We report a few in detail:

Report of Experiments

Case 6.—November 4, 1911. Baby K., 2 hours old. Weight 7 pounds, 9 ounces. Experiment lasting one hour and fifty minutes.

Specimen 1, aspirated after five minutes; 6 c. c. cloudy fluid; moderate amount of saliva; free HCl=56. (Five c. c. tested.)

Specimen 2, aspirated after 10 minutes; 2.5 c. c. cloudy fluid; moderate amount of saliva; free HCl= 50-55. (Two c. c. tested.)

Specimen 3. Catheter withdrawn; reintroduced after ten minutes' interval; aspiration continued intermittently for fifteen minutes. Three c. c. fluid; marked saliva. Free HCl=30. (Three c. c. tested.)

Specimen 4. Catheter withdrawn; reintroduced after twenty-five minutes' interval, and aspiration continued intermittently for fifteen minutes; 25 c. c. fluid; slight saliva. Free HCl=50. (Two c. c. tested.)

Specimen 5. Catheter reintroduced after fifteen-minute interval; aspiration for fifteen minutes; 3 c. c. fluid; slight saliva. Free HCl=46. (Three c. c. tested.)

Epicrisis.—In a test of an hour and fifty minutes 17 c. c. of highly acid gastric juice was obtained from a new-born infant. No decrease in the amount or strength of HCl secretion was evident during that period.

Case 30.—December 4, 1911. Baby B., 1½ hours old. Weight 6 pounds, 8 ounces. 3 p. m., No. 14 F. catheter passed. Gagging slight; 4 c. c. viscid, Congo +++ fluid obtained; saliva marked.

3:05. Catheter reinserted; 3 c. c. Congo +++ fluid. Saliva.

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[10] Ibrahim, J.: Ztschr. f. Biol., liii, 201.
[11] Pawlow, I. P.: The Work of the Digestive Glands, second English ed., 1910.
[12] Cohnheim, O., and Soetbeer, F.:. Ztschr. f. physial. Chem., 1903, xxxvii, 467.

3:15. Inserted into esophagus; saliva and neutral fluid obtained.

3:25. Stomach entered; very slight saliva.

3:30. Stomach contained small amount of Congo +++ juice and saliva.

3:40. Two c. c. Congo +++ fluid with saliva. Baby quiet. Catheter kept steady and not moved about.

3:42. Duodenum easily entered; dry tap after twelve minutes.

3:54. Good retraction test as re-entered stomach. About 1 c. c. clear Congo + fluid. No saliva.

3:55. Re-entered duodenum. Dry tap after five minutes.

4:00. Good retraction test. About 2 c. c. Congo +++ fluid in stomach. No saliva.

Epicrisis.—In a test lasting an hour, free HCl was continuously found in the stomach.

Case 39.—December 28, 1911. Baby U., 5 hours old.

11:55 a. m. No. 14 F. catheter passed. In two minutes 3 c. c. Congo +++ fluid; moderate saliva; pharynx reflex increased; tube washed.

12:00 noon. Catheter introduced into duodenum; marked gagging.

12:10 p. m. Good retraction test; thick mucus. Congo negative; fluid aspirated.

12:15 p. m. Reintroduced into duodenum and retained in place.

12:27 p. m. Retracted into stomach; 0.5 c. c. Congo +, Litmus ++ fluid. Catheter withdrawn.

12:30 p. m. Re-entered stomach; 2.5 c. c. Congo + fluid; some saliva.

12:35 p. m. Reintroduced into stomach; 0.5 c. c. Congo ++ fluid.

Epicrisis.—In a test lasting forty minutes, free HCl was repeatedly aspirated from the stomach.

It is evident, therefore, that whatever may be the nature of the stimulus, not only is hydrochloric acid present in the stomach of the new-born, but that *it may be secreted almost uninterruptedly for long periods* (*hours*) *quite independent of the ingestion of any food.*

Reference has been made to the fact that in one instance there was an absence of hydrochloric acid. It was not until the thirty-fourth case that a result of this nature was encountered.

Case 34.—December 18, 1911. Baby F., 5½ hours old; weight 8 pounds, 8 ounces.

2:30. No. 14 F. catheter introduced; small amount neutral Congo negative fluid; slight blood and saliva; pharynx reflex almost absent.

2:35. Reintroduced. Congo negative; litmus negative.

2:38. Reintroduced; 0.5 c. c. fluid, Congo negative; litmus negative.

2:45. Reintroduced; 0.5 c. c. fluid, Congo negative; litmus negative.

2:55. Entered duodenum easily. Dry tap after twenty minutes.

3:08. Entered stomach; 0.5 c. c. saliva neutral, Congo negative fluid.

Epicrisis.—Absence of hydrochloric acid and neutral reaction of stomach contents in five successive tests during a period of thirty-eight minutes.

Case 34.—Second test.

December 26, 1911; 8 days old; nursed 1¼ hours previously.

3:17. Five c. c. milk aspirated. Congo negative.

3:27. Catheter passed again. Entered duodenum easily.

3:42. Retracted into stomach. Fluid contained saliva, mucus, Congo +.

3:45. Passed into duodenum; 0.6 c. c. fluid containing bile.

4:10. Reintroduced into stomach. During five minutes Congo negative.

Case 34.—Third test.
December 27, 1911; 9 days old; nursed shortly before test.
2:54. No. 14 F. catheter passed. Two ounces of milk obtained. Congo negative, litmus +.
3:00. Duodenum easily entered.
3:10. Retracted into stomach. Congo negative, litmus +.
3:13. Reintroduced. Congo negative.
Epicrisis.—Retests when the infant was 8 and 9 days old showed HCl present but deficient.

Among the fifty-five infants tested, this was the only one in which there was complete absence of hydrochloric acid at birth. The two later tests, detailed above, show that acid was being secreted, although in small amount. We are evidently dealing with a functional disturbance, an anomaly of secretion which may be termed *congenital hypochlorhydria.* Another case (Case 54) should be mentioned in this connection, as it was the only other one in which free acid was not found at birth. However, as the gastric fluid was acid, and the test lasted but five minutes, we must consider that there was merely a deficiency of hydrochloric acid in this case. On the other hand, instances were also met with in which there was a comparative *hypersecretion* of hydrochloric acid; the case (Case 6) cited above as a marked instance of continued secretion may well be brought under this category. We are unable to state the significance of these abnormal variations of secretion. It would be interesting to know how long they persist, or of their possible association with similar conditions in later life.

It is not proposed to consider the gastric secretion of infants who have been put to the breast. However, a few words in this connection may not be amiss, as we carried out a gastric examination on a large group of infants under 10 days of age, incidental to the study of icterus neonatorum. Furthermore, the opportunity was frequently afforded of examining the same infant, first when it was unfed, and later in the course of the first week or two of life. The following case brings out sharply a comparative test of this kind:

Case 39.—First test; infant 5 hours old.
11:55. Catheter passed. In two minutes about 3 c. c. Congo +++ fluid. Moderate saliva.
12:00. Reintroduced. Easily entered the duodenum; mucus.
12:15. Reintroduced into the duodenum.
12:27. Retracted into stomach. Congo +, litmus ++ fluid.
12:30. Passed into stomach again; 2.5 c. c. Congo + fluid; saliva.
12:35. Reintroduced into stomach; 0.5 c. c. Congo ++ fluid.
Second test; infant 6 days old.
3:38. Catheter passed. About 1 ounce fluid, Congo negative.
3:40. Passed pylorus.
3:48. Retracted into stomach; Congo negative fluid.
3:50. Reinserted into duodenum; thick mucus.
4:01. Reinserted. Some mucus, neutral, Congo negative.
4:05. No. 15 F. catheter inserted.
4:25. Withdrawn; alkaline mucus. Congo negative; material in stomach.

Epicrisis.—Test when 5 hours old (unfed) showed marked hydrochloric acid in stomach and continued secretion for forty minutes. Test when 6 days old showed no hydrochloric acid during forty-five minutes.

It is difficult to explain the fact that in this case and in some others there was a greater secretion before the child had been fed than a few days later. We should have expected to find quite the contrary condition, when we consider that at the time of the first test there was an absolute lack of stimulus from food, which is regarded as a powerful gastric secretogogue. We do not refer merely to the fact that more gastric juice was found in the stomach soon after birth than later. This might be accounted for by the supposition that it was the product of many hours of secretory activity. But here is an instance in which the stomach, in the case of an unfed infant, continued to secrete acid juice for forty minutes, whereas in the same infant, after it had been nursed for some days, acid secretion was absent or lacking—in a test lasting for a longer period. Evidently the stimulus to gastric secretion is at times more potent at birth than during the early days of infancy.

Nature of Stimulus of Hydrochloric Acid Production

The consideration of the nature of the stimulus of the hydrochloric acid secretion proved a most interesting part of this study. It seemed adapted to investigation, although we realized at the outset that it would be hampered by the limitations which surround all physiologic studies in human beings. On the other hand previous studies of this nature are open to the criticism that they have been carried out on animals, and that their deductions have been presumed to apply to human physiology. But before proceeding further, it may be well to outline the present viewpoint in regard to the normal mechanism of the secretion of gastric juice. Let us follow a standard text-book of physiology.[13] In the first place, it is considered a proved fact as the result of Pawlow's experiments, that "mechanical stimulation of the gastric mucous membrane has no effect on the secretion of the tubules." There are three steps in the mechanism of scretion: (1) The psychical secretion, for which the afferent stimuli originate in the mouth and nostrils, and the efferent path through the vagus nerve; (2) the secretion from secretogogues contained in the food; (3) the secretion from secretogogues contained in the products of digestion. The manner in which the secretogogues act cannot be positively stated. Recently Edkins[14] has suggested, as the result of experiments. that a gastric secretin is formed through the interaction of the food and of the mucous membrane of the pylorus; that this substance is absorbed by the blood, and, acting as a hormone or messenger, is carried to the gastric glands, stimulating them to secretion. According to these views, as given by Howell, we must distinguish between a nervous secretion due to the action of the secretory fibers in the vagus, and a chemical secretion due to the chemical stimulation of secretogogues or hormones. Viewed from this physiologic standpoint, how are we to interpret the fact that (a) hydrochloric acid and gastric ferments are found in the stomach soon after birth, and (b) that the new-born infant, although unfed, continues to secrete them?

It is at once evident that prevailing physiologic views do not account for (a) the secretion of hydrochloric acid immediately after birth. For the exciting stimulus can be neither psychical, "a term which implies that the reflex must be attended by conscious sensations," nor, as we are considering unfed infants, can it be chemical, a secretogogue formed or stimulated by the food.[15] Although this is evident, and it is clear that some other mechanism must exist to account for the secretion at this time of life, we are not prepared to offer a new hypothesis. There are some factors, however, associated with this problem which are worthy of further consideration and discussion. It will be seen from the table (Table 1) that a juice containing hydrochloric acid was obtained as early as one-half hour after birth, and that it was frequently found within the first two hours of life. This secretion may be regarded as either prenatal or postnatal in origin. If the latter view be accepted, we may regard it as in some way connected with the changes in the respiratory and in the circulatory systems, which come about at the time of birth. On the other hand, it may well be that the juice was secreted before birth as the result of an unknown mechanism different from that which obtains in extra-uterine life. This conception of the prenatal secretion of gastric juice, containing hydrochloric acid and ferments, at once raises the question of a fetal gastric digestion, an interesting but at present a purely speculative field.[16]

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[13] Howell, W. H.: Text-Book of Physiology, 1911, p. 763.

[14] Edkins: Jour. Physiol., 1906, xxxiv, 133.

Let us now turn to the other phase of the problem (b), the continued secretion of gastric juice by the unfed infant. We have stated that likewise this secretion can be neither of reflex origin, that is to say, psychic, nor of chemical origin, namely, due to a secretogogue. In the course of the many tests on new-born babies, observations were noted regarding the effect of mechanical irritation of the stomach, the secretion of saliva, the sucking of the tube by the infant, and other factors, in order to ascertain whether any parallelism could be discovered between these possible stimuli and the degree of gastric secretion. It should be remembered that throughout extended tests we obtained gastric juice containing a high degree of hydrochloric acid. The instances which have been cited above, in which the juice was obtained for twenty minutes, forty minutes, or even for an hour or more are not exceptions. It seemed as if we would have ample opportunity to study this question. However, in most instances the phenomena were interwoven and could not be isolated; the infant which sucked the catheter had also a flow of saliva, or, for example, where mechanical irritation was attempted, some secondary factor could not be rigidly excluded. Nevertheless, some cases are worthy of note.

It has been suggested that the saliva may act as a stimulus to the secretion of gastric juice. From this point of view it is significant that, although at times both salivary and gastric secretion were profuse, in the only case (No. 34, cited above in detail) in which no hydrochloric acid whatsoever was found, there was an average quantity of saliva. In another instance (No. 35) hydrochloric acid was obtained on the introduction of the catheter, but could not be found later in the test, although salivation was marked and aspiration was continued at intervals for half an hour. We select these two cases for illustration as they seem conclusive, unless we are to consider them as exceptions to a rule. If not, we must conclude that the saliva does not constitute the essential gastric stimulus in the new-born.

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[15] In this connection mechanical stimulation may be left out of consideration, as in many instances the catheter was rapidly passed and the gastric contents obtained immediately on its introduction. A latent period of less than a minute between a mechanical stimulus and profuse secretion is inconceivable and contrary to all experience.

[16] Although analogies cannot serve as arguments, we may recall in this connection the prenatal secretion of the liver (bile), of the sebaceous glands (vernix caseosa), and of the glands of the vagina. It should also be considered whether the infant in utero does not perhaps swallow liquor amnii oftener than is generally believed, and whether this fluid may not act as a stimulus to gastric secretion.

We were not able to control the gastric secretion by mechanical stimulation, that is, to increase it by moving the catheter about in the stomach, or to decrease its flow by maintaining the catheter at rest. But it should be added that as each case differed in the amount of gastric secretion, it would be unwise to draw deductions from individual instances. It seemed as if the secretion of mucus rather than of hydrochloric acid was stimulated by mechanical irritation.

Influence of Sucking on Gastric Secretion

From the beginning our attention was directed to the influence of sucking on gastric secretion. Some years ago Pfaundler[17] studied this question clinically, and suggested that there is more acid secreted when infants nurse actively than when they are fed passively. The lack of normal standard for gastric juice rendered his figures inconclusive. Cohnheim and Soetbeer[12] investigated this subject on very young puppies, on which an esophagostomy and a gastrostomy had been performed; however, the animals did not live long. The authors came to the somewhat hypothetical conclusion that secretion is a congenital reflex. We approached the question clinically by having the infant suck on a catheter passed as far as the esophagus; this allowed the saliva to be continuously aspirated as it flowed down from the pharynx, but did not entail mechanical stimulation of the stomach. After holding the catheter in this position for a definite length of time, it was rapidly passed into the stomach and the gastric juice obtained. In almost every test of this nature we found hydrochloric acid. However, we were not able to establish a direct relationship between the length of time of sucking and the quantity of gastric juice. We could not demonstrate that the amount of hydrochloric acid was regularly greater in tests of one-half hour than in those of one-quarter hour, nor could we regularly produce still larger quantities in longer tests. This may have been due to congenital differences of secretion among the various infants, which, as we have shown, exist. Therefore, although our results were not sharp and definite, we believe this method of studying this question on infants deserving of further trial.

In adults the hydrochloric acid of the gastric juice, in addition to its direct function of digestion, is regarded as playing a large part in the regulation of the patency of the pyloric sphincter, and, indirectly, in the secretion of pancreatic juice. Let us briefly consider its relationship to these two phenomena at this early period of life. It has been noted elsewhere that at birth a catheter may readily be inserted through the pylorus into the duodenum. In fact, it can be passed on the new-born with greater facility and with less force than at almost any other period of infancy. Some fifty duodenal tests, made soon after birth, for the purpose of taking cultures, or of obtaining bile or pancreatic ferments, convinced us of this fact. We can therefore state with conviction that a marked tightening of the pyloric sphincter was not associated with the chlorhydria of the new-born; that there was no parallelism between these secretory and motor gastric phenomena. To further test this observation we introduced, in several cases, 1 or 2 c. c. of 0.4 per cent. hydrochloric acid and then passed the catheter. We do not wish to enter into this subject in detail, as we shall consider it at another time in connection with pylorospasm; however, we may summarize these experiments, from the present point of view, with the statement that even following the introduction of the free acid we were able to traverse the pylorus. This accords with the view of most physiologists that hydrochloric acid does not incite closure of the pylorus while it is on the gastric side of the sphincter. This does not, however, exclude the possibility that there may be a relationship between congenital gastric hypersecretion and the development of pylorospasm or of duodenal ulcer, two conditions somewhat peculiar to infancy.

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[17] Pfaundler, M.: 16 Verhandl. d. Gesellsch. f. Kinderh., 1899, p. 38.

Relationship of Gastric and Pancreatic Juices

Finally, a word should be added concerning the relationship of the gastric and the pancreatic juices. In the adult the main stimulus for pancreatic secretion is regarded as the gastric juice, indirectly through its hormone action. In the unfed new-born infant the relationship does not seem to be so intimate. At this time of life it was at all times difficult to obtain pancreatic juice; in many instances no fluid could be obtained; in others but a fraction of a cubic centimeter. There was a striking disproportion between the large amount of gastric juice and the small quantity of secretion in the duodenum. This lack of concordance may be explained by the supposition that the hydrochloric acid remains in the stomach and does not gain access to the duodenal mucous membrane. Whether or to what extent this is true we have no means of judging. However, in many cases gastric juice entered the duodenum in the course of the passage of the duodenal catheter; in others natural or artificial gastric juice was instilled into the duodenum. Nevertheless, we failed to stimulate pancreatic secretion. In similar tests performed on somewhat older infants we produced at will by this means an increased flow of pancreatic fluid. It is evident, therefore, that at birth the pancreatic secretory mechanism is either functionally undeveloped, or needs a factor in addition to hydrochloric acid to set it in action.

Conclusions

New-born infants regularly secrete a considerable amount of hydrochloric acid before they are given any food. Among fifty-two infants varying in age from one-half hour to eighteen hours, only one did not have hydrochloric acid in the stomach; in all but one instance free acid was obtained.

The hydrochloric acid varies greatly in amount. Exceptionally it was found almost lacking on repeated tests (congenital hypochlorhydria or hyposecretion), or very profuse (congenital hyperchlorhydria or hypersecretion).

In almost all cases acid was obtained throughout prolonged tests, in spite of the fact that food was not given to stimulate secretion. In one instance 17 c. c. of highly acid juice was aspirated in one hour and fifty minutes. Rennin, pepsin and lipase were also obtained in the (unfed) new-born.

Prevailing physiologic views cannot account for the gastric secretion immediately after birth. It is not the result of mechanical stimulation by means of the catheter, as the juice was obtained immediately on the introduction of the tube, without an intervening latent period. It may be prenatal in origin. Nor is it clear what stimulates the continued secretion which was obtained for hours. Experiments showed that the saliva is not the exciting agent; the effect of sucking could not be determined. Comparative tests of the same infants at birth and later, during the first week of life, showed that the stimulus to gastric secretion may be greater in the new-born infant which has not been fed.

This chlorhydria of the new-born is not usually associated with increased tonicity of the pyloric sphincter, as the duodenal catheter can readily be passed through the pylorus. Even when 0.4 per cent. hydrochloric acid is instilled into the gastric cavity the catheter can be readily passed into the duodenum. However, the high acidity may at times be related to the pylorospasm or to duodenal ulcer met with in infancy.

Although gastric secretion is so marked in the new-born, duodenal and pancreatic secretion is very scanty. Nor can this secretion be readily stimulated by allowing hydrochloric acid to enter the duodenum. Evidently the mechanism of pancreatic secretion is not as easily activated in the new-born as in later infancy.

SIGNIFICANCE AND PREVENTION OF AMEBIC INFECTIONS IN THE MOUTHS OF CHILDREN

Anna Wessels Williams, Anna I. von Sholly, Caroline Rosenberg and Alice G. Mann

From time to time during more than half a century, a protozoan genus called *Ameba* by some, and *Endameba* or *Entameba* by others, has been reported as occurring chiefly about the gum margins in the oral cavity of human beings.

Few details of the morphology, comparative incidence and pathogenicity of the members of this genus have been given. Until last year, most, if not all of the species, were thought to be non-pathogenic. Indeed, of so little worth were they considered, that no mention of their presence is made in the general histological review of parasitic amebas of man by several authors, who used the term *parasitic amebas* as synonymous with *intestinal amebas.*

Thus Craig says—"There is no experimental evidence connecting *Ent. buccalis* (or the mouth ameba) with disease. It is very doubtful if it has anything to do with caries of the teeth, although it is most frequently encountered in the cavities of carious teeth. However, it may be frequently demonstrated in material scraped from the roots of perfectly normal teeth, so that, as far as the evidence goes, we must regard *Ent. buccalis* as only a secondary invader. The amoebae known as *Ent. gingivalis,* Gross 1849, and *Ent. dentalis,* Grassi, 1879, are probably identical with *Ent. buccalis* and do not merit separate description."

In 1914 Smith and Barrett, followed by Bass and Johns and several other investigators, claimed that mouth amebas occurred chiefly in cases of pyorrhoea alveolaris or Riggs disease, and that they were the probable cause of this common septic infection of the jaws. Other observers have not agreed with this claim because sufficient evidence has not been given by its authors to make us sure of the types of amebas found and of the extent, if any, of their pathogenic action. So several hypotheses may be formulated as to the part thought to be played by amebas in mouth infections.

Hypotheses in Regard to Action of Amebas in Human Mouths

1. Amebas are accidental invaders, they produce no pathogenic or other reaction in the mouth. (Provazek, Hartmann and others.)
2. Amebas in the mouth have a beneficent action as scavengers. (Chiavero and others.)
3. Amebas may be sometimes pathogenic, producing certain cases of pyorrhoea alveolaris, certain abscesses and certain other mouth infections. (Leidy, Gross, Flexner, Smith & Barrett and others.)
4. Amebas are the specific cause of pyorrhoea alveolaris. (Bass and Johns and others.)
5. Amebas may be only pathogenic in connection with certain other microorganisms.

The comparative worth of these hypotheses may be discussed as follows:

1. In favor of the first hypothesis are the facts that amebas have been reported so frequently in apparently normal mouths and that when found in diseased conditions, they are always accompanied by other microorganisms of pathogenic types, which might be sufficient to produce the disease. Against it is the fact that they are found in so much larger numbers in the lesions.

2. There is no question but that amebas are scavengers both of other microorganisms and of tissue host cells—at least, of leucocytes, red blood cells and broken down epithelial cells. But they probably take up as many harmless as well as harmful germs and leave enough of the latter to exert their full power in harming the tissues of the host. Then, too, since we know that certain amebas will live on brain, liver and kidney cells of humans, there is no *a priori* reason to suppose that the mouth amebas will not help in destroying the fixed tissue cells in the mouth, especially after a certain amount of injury has been done them by other microorganisms. The evidence then is against this second hypothesis.

3. The large number of amebas in most cases of pyorrhoea and in some abscesses of the jaw, and their infrequent presence in absolutely healthy mouths, makes tenable the third hypothesis.

4. Whether or not they are the specific cause of pyorrhoea can only be determined after further study. Some facts brought forward in this paper bring new evidence on this point.

5. We must remember that if amebas absorb and digest fixed tissues as well as leucocytes, blood cells and other wandering cells, they may help decidedly in keeping up a lesion bacteria have started. This fifth hypothesis is also tenable.

ORIGINAL WORK

In order to help determine which, if any, of these hypotheses is correct, we have been trying to find out *first,* how many children are infected and at what age the amebas begin to appear in their mouths as well as to study the condition of the mouths showing the amebas. *Second,* what types, if more than one of amebas, are present. *Third,* the relation, if any, of these amebas to the diseased condition of the children's mouths, and *fourth,* the worth of preventive measures.

We have begun our study by examining the mouths of about one thousand school children in one of the poorer districts of New York City. We have already started the examination of a similar group in one of the more well-to-do districts.

At the same time, the Bureau of Laboratories of the New York City Health Department has begun a more general study of focal mouth infections, particularly of pyorrhoea alveolaris.

Soon after our observations on adults were begun, we found that all cases presented to us by the several dentists who are collaborating with us, already had pus pockets formed in the jaw alveoli—that is, they were well-established cases—and when we began to inquire for beginning cases, we learned that the diagnosis of pyorrhoea alveolaris is not made by most dentists until pus pockets can be demonstrated, so this added interest to our decision to hunt for beginnings in school children.

So far 1678 children (those in only one session of the school) between the ages of 5 and 16 years have had a preliminary examination, and from 995 representative ones stained smears have been made and examined for amebas. The 1678 children have been divided roughly into those having healthy gums and those with receding or spongy and bleeding gums. The healthy looking gums were further divided into those with one or more decayed teeth, and those with apparently sound teeth. Needless to say, we found very few of the latter, that is, of those with apparently healthy gums and sound teeth. About one-half showed unhealthy gums.

Results of Smear Examination of 995 *Cases.*—From many cases two smears were taken. The smears were made as follows: In the bad cases, the teeth and gums were cleaned with a cotton swab dipped in 50 per cent. alcohol, then with the flat end of a hard wood sterile tooth pick, material was scraped from the margin of the gums and stroked lightly over a clean glass slide making 3 or 4 streaks. The smear was then fixed while still moist, by dropping methyl alcohol on it, and it was ready to send to the Laboratory. This was called the superficial smear. A second tooth pick was used to get material from beneath the margin of the gums, from which another smear was made and fixed. This was called the deep smear. In the cases with clean teeth and tight healthy looking gums, the first smear was made before the cleaning with alcohol. All of these smears were stained with Giemsa's stain and examined for amebas. In regard to the identification of amebas by stained films, we would say that they are more certainly and easily identified by this method than by warm living preparations, though, of course, both methods should be used in studying all of the characteristics of these microorganisms. In hunting for amebas in these smears we have found that most of them seem to be between the superficial bacterial layer and the deep leucocytic layer, so one may find them only in large numbers in one small part of a smear, especially in a smear taken in a routine way.

Stained by Giemsa, the delicately but definitely reticulated cytoplasm of the ameba stains a clear blue, while the small nucleus shows as a delicate rounded or irregular mass of red granules and threads, with often a central light blue staining karyosome, sometimes containing a vacuole-like body. This nucleus no doubt, corresponds to that most frequently described for "*Entameba histolytica-tetragina.*" There has been found no marked variation from this type of nucleus.

Amebas are so notoriously variable in their entire morphology, according to their stage of development, and to the conditions of growth, that no definite opinion should be expressed as to the species of the forms found in these mouths until much further study has been made. We may say, however, that there seem to be at least two morphyological types of amebas present—one small, with more or less rounded, dense nucleus, with few or no inclusions or vacuoles found in the majority of healthy mouths, and the other large, with more irregular nucleus with many large vacuoles and containing many red blood cells, leucocytes and other dense masses formed by undigested material. In the majority of unhealthy mouths the appearance of this latter type agrees with that of the type most frequently seen in the well developed cases of pyorrhoea alveolaris.

NUMBER AND PERCENTAGE OF AMEBAS FOUND IN EACH GROUP OF CHILDREN

Between 5-16 Years			Between 5-7 Years		
Condition of Mouth	Total	Amebas Present	Condition of Mouth	Total	Amebas Present
I. Healthy Gums...	+ 38 − 92	29 Per Cent.	Healthy Gums...	+ 3 − 31	9 Per Cent.
II. Healthy Gums... Decayed Teeth...	+109 −183	37 Per Cent.	Healthy Gums... Decayed Teeth...	+ 32 −105	23 Per Cent.
III. Tartar and Receding Gums.....	+182 − 96	65 Per Cent.	Tartar and Receding Gums...	+ 19 − 17	53 Per Cent.
IV. Spongy Bleeding Gums.........	+268 − 28	91 Per Cent.	Spongy Bleeding Gums.........	+ 34 − 5	70 Per Cent.

We have found that amebas begin to appear at a very early age, 35 per cent. of children between 5 and 7 years of age being infected. The numbers of those infected with amebas increase in frequency with age. Between 5 and 15 years, 60 per cent. show these protozoa on their gum margins. In apparently normal mouths only 29 per cent. show amebas.

Finding the amebas so often in apparently healthy mouths and in such young children, does not agree with the statement of Bass and Barrett that they are not found in healthy mouths. Whether or not one or more varieties are taken from adult pyorrhoea cases and are simply waiting their opportunity to exert a pathogenic action, we cannot yet say. But the evidence seems to point toward the establishment of either the first or the fifth hypothesis already given.

These results, however, as they stand are very striking, especially when we must add that most of the cases of spongy and bleeding gums show more amebas in the films than the other cases. If we could only rule out other micro-organisms, the evidence for the pathogenic action of the amebas would be stronger.

We have, in these school children carriers of amebas, a marked opportunity to test the prophylactic effect of ipecac or its emetin alkaloid on mouth amebas.

We have divided the cases showing amebas into 3 groups, one to be watched without treatment, and others to be treated in different ways, one with emetin and the other by ordinary cleanliness. We have a tooth wash made up containing a dilution of emetin, and have the washing of the teeth done morning and afternoon in the school clinic under the supervision of the school nurse.

The first examinations, one week after the beginning of this treatment, gave marked results, in that nearly every case receiving emetin showed no amebas. In the later examinations, however, amebas were found in a few of the emetin-treated which were negative before, while a few more of the controls showed none.

The following points have been brought out so far by these examinations:

1. Amebas are irregularly demonstrated in all mouths once showing them, most constantly and in largest numbers in mouths showing gingivitis, least so in healthy mouths.

With ordinary teeth cleansing methods, the number of mouths showing amebas is reduced one half. With emetin in the tooth wash, the number showing amebas is greatly reduced—only about 10 per cent. showing them. The second set of controls—those doing their cleaning *at home* by ordinary methods—continue to show amebas in about 75 per cent. of the cases.

The question as to the amount of emetin to be used has not yet been settled. We began with a 1-200 solution, then reduced to a 1-400 strength, Probably a much smaller amount would be sufficient to keep down the development of the amebas.

We hope to carry on these investigations for several years and if necessary, to extend the number of children included in the groups. Whether we can demonstrate fully the efficiency of the use of emetin in preventing many cases of pyorrhoea alveolaris, remains to be seen. We hope by combining these practical investigations with the laboratory studies that we may be able to help demonstrate the relationship of these amebas to septic mouth infections.

THE COMPLEMENT FIXATION REACTIONS OF THE BORDET-GENGOU BACILLUS

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Serological studies of the relationship of the Bordet-Gengou bacillus to hemoglobinophilic bacilli have been made by various investigators since Bordet and Gengou, in 1906, claimed the discovery of the etiological cause of whooping cough in the bacillus bearing their name.

In their first article on the subject[1] Bordet and Gengou stated that an influenza antigen used in tests on the serum of pertussis convalescents (which with an antigen of the Bordet-Gengou bacillus invariably inhibited hemolysis) did not inhibit hemolysis, from which they concluded that the influenza bacillus has no etiological relationship to whooping cough and that the complement fixation test could be utilized to differentiate these two bacterial species. They found the antigen of the influenza bacillus to be anticomplementary but not the antigen of the Bordet-Gengou bacillus. Arnheim[2,3] also tested the serum of pertussis cases with both Bordet-Gengou and influenza antigens, and with the latter all results were negative. In working with immune rabbit serums he found that deviation in the presence of homologous antigens always took place even in small amounts of antibody and that Bordet-Gengou and influenza strains did not cross-fix. Wollstein,[4] working with immune rabbit serums, found the serums of rabbits immunized with various strains of the Bordet-Gengou bacillus to fix complement in the presence of Bordet-Gengou antigen but not influenza antigen, while influenza immune serum bound complement with an influenza antigen and not with the Bordet-Gengou antigen. Baecher and Menschikoff[5] successfully used the complement fixation reaction to confirm the identity of freshly isolated strains of the Bordet-Gengou bacillus from the influenza bacillus. Shiga, Imai and Eguchi[7] state that the Bordet-Gengou bacillus can be clearly differentiated from influenza bacilli by means of complement fixation. In testing the serum of rabbits immunized with the Bordet-Gengou bacillus, they never obtained complete inhibition with an influenza antigen; but in testing influenza serum they did in some instances, not in others, obtain complete inhibition with a Bordet-Gengou antigen, though not in so high a dilution of serum as with an influenza antigen. Inaba[8] described antibody content titrations of the serum of five rabbits immunized with five strains of the Bordet-Gengou bacillus. The serums were tested against antigens of ten strains of the Bordet-Gengou bacillus and practically the same amount of inhibition occurred in all tests. Winholt[9] has recently reported on the absence of cross-fixation between the Bordet-Gengou bacillus and the influenza bacillus.

The objects of our investigation were to confirm the above results concerning the differentiation, through complement fixation tests, of the Bordet-Gengou bacillus from hemoglobinophilic bacilli, to differentiate the organism from certain forms, culturally and morphologically atypical, obtained from the sputum of pertussis cases and to determine the inter-relationship of the various strains of the Bordet-Gengou bacillus. Work on the serums of pertussis cases has been described in another article.[10]

Strains.—Fourteen strains of the Bordet-Gengou bacillus have been used in this work, four strains of the Bordet-Gengou bacillus morphologically and culturally atypical, and nine strains of hemoglobinophilic bacilli. The Bordet-Gengou strains P.D. and M and the hemoglobinophilic strain BI_2 were obtained from other laboratories. The hemoglobinophilic strains 747, 11, and z were isolated from the spinal fluid of cerebro-spinal meningitis cases. All the other strains were obtained from the sputum of pertussis cases. Most of the strains were isolated and identified, morphologically and culturally, by Dr. A. W. Williams, who, in regard to strains isolated from whooping cough cases, has stated:[11] "The majority of the strains isolated by us have been shown to be a distinct species from the hemoglobinophilic bacilli and from certain other bacilli morphologically similar to the Bordet-Gengou bacillus. Not only are cultural characteristics different, but these strains produce specific antibodies in experimental animals which give positive results with the complement fixation test."

Technic.—The volume of all our complement fixation tests is one-tenth that of the classical Wassermann, .5 cubic centimeter instead of five cubic centimeters. For a hemolytic system we have used sheep cells in a five per cent. suspension, rabbit amboceptor and guinea-pig complement in a ten per cent. dilution. The balance of the system has been daily obtained by means of an amboceptor titration with .1 cubic centimeter of ten per cent. complement incubated one hour in a water bath at 37° C.; and an amount of amboceptor between one and two units thus determined has been used to sensitize cells for filling antigen and antibody content titrations, the exact amount of amboceptor depending on the rapidity of hemolysis, the amount hemolyzing in about thirty minutes being chosen.

The relationship of strains has been determined by means of antigen and serum titrations. (The technic of titrations is that described in "Pathogenic Micro-organisms," by Park and Williams, 1914, pp. 184-187.) All titrations have been incubated in a water bath at 37° C. one-half hour before the addition of sensitized cells, one hour afterwards. The readings have been made after the settling of the cells, usually the following day, the tests meantime standing in the ice-box. If an immediate reading was desired, the tubes were centrifugalized. All serum has been freshly inactivated before use by heating one-half hour at 56° C. Each antigen has been standardized by titrating with a previously standardized homologous immune serum, so diluted that .01 cubic centimeter contained about two antibody units. If no serum of the same strain was available, serum of some closely related strain was used. When neither antigen nor serum was standardized, the antigen was first titrated with .01 cubic centimeter of undiluted serum, and with the antigen unit thus obtained the serum was titrated. After obtaining the serum unit the antigen was re-titrated with a suitable amount of serum. Each antigen has been tested in a sufficient number of dilutions (1 in 10, 1 in 100) to determine the smallest amount that with a minimum amount of immune serum completely inhibits hemolysis; this amount we designate as the Antigen Unit. Immune serum has been titrated with antigen so diluted that .1 cubic centimeter contained about two antigen units, according to standardization with homologous serum. Each serum has been tested in a sufficient number of dilutions (1 in 10, 1 in 100) to determine the smallest amount that with a minimum amount of antigen completely inhibits hemolysis; this amount we designate as the Antibody Unit.

Antigens.—Antigens have been prepared according to various methods. The media used were 1-500 coagulated horse blood veal agar (blood added to agar at 90° C.) for influenza and older pertussis strains, Bordet-Gengou for recently isolated pertussis strains and salt free veal agar, neutral to phenolphthalein, for the atypical Bordet-Gengou strains. In the beginning of the work a modification of Schwartz and McNeil's method of preparing gonococcus antigen was employed—a forty-eight-hour growth (on neutral, salt free veal agar) in pint blake bottles was washed off with neutral distilled water, heated in a water bath at 56° C. for two hours and filtered through a Berkefeld. This method of autolyzation proved to be satisfactory for atypical Bordet-Gengou strains and fairly so for the Bordet-Gengou, especially if the growth was scraped off with a platinum spud and deposited in distilled water, thus preventing the extraction from the medium of substances that render an antigen non-specific. As, however, the pertussis organisms are not easily autolyzed, the extracts thus obtained were weak. Shaking for two to eighteen hours was found to add greatly to the efficiency of pertussis antigens. Of the various combinations of shaking and heating that have been tried the following has given the best results and is now used in the preparation of all pertussis antigens: A forty-eight-hour growth on Bordet-Gengou medium is scraped off and deposited in neutral distilled water and put in an electric shaker for three to four hours. The temperature is then brought up to 56° C. in a water bath and the emulsion is left in a thermostat at 56° C. overnight. The following day it is centrifugalized and the supernatant fluid or Berkefeld filtrate is used as antigen, after being made isotonic by the addition of nine per cent. saline.

In determining the comparative value of different antigens, the range of fixation with homologous immune serum—*i. e.,* the difference between the anticomplementary dose and the antigen unit—and specificity are the chief points to be considered. As far as specificity is concerned, the different methods of antigen preparation that have been tried have given practically the same results. There is such great individual variation in antigens of the same strain made by the same method that a comparison of methods is difficult. If the value of an antigen is expressed numerically a comparison is facilitated. The scheme suggested by Walker and Swift[12] is convenient. If the anticomplementary dose is divided by the antigen unit, a figure is obtained that may be called the Index of fixation. The larger the index the stronger the antigen, hence the method of preparation is best for the production of a strong antigen that has the highest median index, *e. g.,* one antigen of strain P.D. made March 25, 1914, autolyzed by heat only, had an anticomplementary dose of .2 cubic centimeter and an antigen unit of .05 cubic centimeter, so the fixation index was $\frac{.2}{.05} = 4$. The same method used before, on October 29, 1913, on the same strain, resulted in antigen with a unit of .015 cubic centimeter and not at all anticomplementary in .4 cubic centimeter, the largest amount tested. The index of this antigen was therefore $\frac{.4+}{.015} = 26.6+$. Another P.D. antigen made on April 4, 1914, autolyzed by shaking two hours and heating at 56° C. one hour, had an anticomplementary dose of over .4 cubic centimeter (exact amount not determined) and a unit of .005, *i. e.*, an index of $\frac{.4+}{015} = 80+$. If the fixation indices of all antigens made by each method are determined, the median index may be taken as a basis for comparison. The Bordet-Gengou antigens extracted by heat only have a median index of 8+, those extracted by shaking and heat have a median index of 26+; thus for the preparation of Bordet-Gengou antigens a combination of shaking and heating seems preferable to heating alone. For the preparation of influenza antigens neither method is really satisfactory. A number of antigens have had so short a range as to be useless. The shaken antigens have been more anticomplementary than the unshaken and have had a median index of 2 only. Such an antigen is too poor to be reliable and has not been used in cross-fixation tests. Most of the influenza antigens autolyzed by heat only have not been anticomplementary in .4 cubic centimeter and the median index is 4+, which indicates a fairly good antigen. To obtain reliable results in any complement fixation work an antigen should be used that has an index at least of 3. Results are likely to be more specific if the fixation index is high. As to the antigens made from the atypical strains, not enough have been shaken to demonstrate the value of that method, but extraction by heat has produced antigens with a median index of 30, which is perfectly satisfactory. It is evident that each organism to be used in complement fixation work requires special study as to the preparation of antigen. The methods used in this work we do not consider ideal and modifications are constantly being tried. The antigens made, however, have been sufficiently satisfactory for at least a preliminary study of the complement fixation reactions of the Bordet-Gengou and allied organisms. By the use of more refined methods it might be possible to obtain antigens so specific that strains apparently identical would be differentiated.

Immune Serum.—Immune serum was obtained by inoculating rabbits with B. pertussis, typical and atypical strains, and B. influenzæ. The injections were chiefly with living forty-eight-hour cultures intraperitoneally, a few with vaccines and antigens subcutaneously and intravenously respectively. Trial bleedings were made before inoculations and about once a week after the third inoculation, until the serum showed a high enough titer for complement fixation tests. The dosage began with the ¼ to ⅛ slant of recently isolated cultures and ½ slant of older cultures. Some of the atypical strains were very virulent and the first dose was from 1/100 to 1/50 slant. The dose was increased according to the weight of the animal. As a rule the increase was gradual until the rabbit received the surface growth from four to six one hundred cubic centimeter bottles. The intraperitoneal injections were once a week, the subcutaneous and intravenous twice a week. The rabbits were bled to death on the seventh or eighth day after the last inoculation.

Of one hundred and four rabbits tested while normal, six only have shown complete inhibition in .02 cubic centimeter, the maximum amount of serum tested. One of these had a unit of .005 cubic centimeter, increasing to .003 cubic centimeter after three inoculations of B. pertussis, one a unit of .006 cubic centimeter, increasing to .003 cubic centimeter after three inoculations of B. pertussis, one a unit of .008 cubic centimeter, increasing to .005 cubic centimeter after three inoculations of B. pertussis, and the other three a unit of .02 cubic centimeter. Of the last group the antibody content increased to .002 cubic centimeter in one instance after three inoculations of B. pertussis; the other two rabbits (inoculated with B. influenzæ) showed no increase in complement fixing substances after three inoculations.

Eighty-one rabbits have been immunized by the live culture method, with an average of eight inoculations. One of these never showed complete inhibition in even .02 cubic centimeter of serum. One had an antibody unit of .0002 cubic centimeter after nine inoculations. If each serum is considered at the time when the antibody content was highest the median unit is found to be .002 cubic centimeter.

To determine the rate of increase in complement fixing substances following inoculations of live cultures of pertussis, atypical pertussis and influenza bacilli, the median antibody unit has been determined for each inoculation. After one and two inoculations no trial bleedings were made. After three inoculations fifty-two rabbits were bled and the serum was titrated against homologous antigens or those most closely related. When a serum has been tested against several homologous antigens, slightly different results have sometimes been obtained, for no apparent reason; and to exclude the error arising from a choice of records, all such tests have been included in determining the median. Thus the number of titrations cited in Table I exceeds the number of serums titrated. Experience has shown that serum with an antibody unit of .003 cubic centimeter is sufficiently strong to give specific results in cross-fixation tests and to retain its complement fixing substances for a considerable length of time, hence such a serum is considered good.

TABLE I.

Complement-Fixing Value of Immune Rabbit Serum After Various Inoculations with Live Cultures of Bordet-Gengou—Atypical Bordet-Gengou and Influenza Bacilli.

Number of Inoculations	Titrations	Median Unit Greater than	Number with Unit of 0.003 c. c. or Less i.e. Good	Percentage Good
3	70	0.02 c. c.	14	20.
4	96	0.004 "	45	46.8
5	108	0.004 "	45	41.6
6	116	0.004 "	53	45.
7	92	0.004 "	41	44.5
8	137	0.004 "	59	43.
9	68	0.003 "	45	66.1
10	58	0.002 "	37	63.7
11	40	0.002 "	31	77.5
12	16	0.0025 "	11	68.7
13	15	0.002 "	8	53.3
14	9	0.0009 "	8	88.8
15	2	0.00055 "	2	100.

If all the good serums are added, according to the number of inoculations, the percentage of good serums resulting from each inoculation is obtained (Table I). According to both the median unit and the percentage of good serums there is a great increase in the development of complement fixing substances after the fourth inoculation. From the fourth to the ninth inoculation little change occurs. With about the ninth inoculation there is another increase, which gradually continues. The number of serums tested beyond the thirteenth inoculation is too small to warrant conclusions. It appears, therefore, that four inoculations are likely to result in the production of a good serum.

Eleven rabbits were immunized with vaccine, ten inoculations, on an average. The weakest serum showed no complete inhibition in .02 cubic centimeter, the strongest showed complete inhibition in .0006 cubic centimeter, the median unit was .007 cubic centimeter. Three rabbits were immunized with the bacterial extract antigens, with an average of six inoculations. The weakest serum had an antibody unit of .005 cubic centimeter, the strongest an antibody unit of .002 cubic centimeter, the median unit was .004 cubic centimeter. From so small a number no conclusions can of course be drawn. There is no doubt but that the live culture method employed is superior to the vaccine method for the production of complement fixing substances.

The immune serums have been preserved by freezing and within a period of one year little change in the antibody content has been observed.

Cross Titrations.—The cross titration work has extended over a considerable period. In working with so many strains it has not been feasible to have on hand at one time satisfactory antigens and satisfactory serums of all strains. Thus no one serum has been tested against antigens of all strains, nor has any one antigen been tested against serums of all strains.

TABLE II
RESULTS OF ANTIGEN TITRATIONS

Antigen Strains		Serum Strains																						
		Bordet-Gengou												Atypical				Hemoglobinophilic						
		PD	M	55	81	93	95	98	100	110	114	121	154	C	I	31	10	33	35	37	87	747	B1$_2$	Z
Bordet-Gengou	PD	+	+	+	+	+	+	+	+		+	+		=	±	±	±		−			±	−	
	M	+	+												±	±		−	−				−	
	55	+	+	+	+	+	+	+	±		+	+		−	−	−	±		−			±		−
	81	+	+	+	+	+	+	+	+	+	+	+				−		−	−				−	
	93	+		+	+	+	+	+	+		+	+				+		+				+	−	
	95	+	+	+	+	+	+	+	+		+	+	+		±	+	±	−	−			±		
	98	+	+	+			+	+	+		+	+		−	+	+	−		−			+	−	
	100	+		+												±		+					−	
	110	+	+		+		+																	
	114		+				+		+		+											+		
	121	+	+	+	+	+	+	+			+	+	+				−				−	+	−	
	141	+		+	+	+	+	+	+		+	+										+		
	154	+	+	+	+	+	+	+	+			+	+	−	±	+	−			−	−	±	−	−
	155	+	+	+	+	+	+	+			+	+	+								−	±	−	
Atypical Bordet-Gengou	C	−	−	−			−	−	−		−			+	−	−	−	−	−			−		
	1	±	−	−	−	−	−		±			±		−	±	±	−					−	−	−
	31	±	±		±	−						±		−	±	+	±	−	−			−	−	−
	10	−	−	−			−	−	−	−		−		−	−	−	+	−	−	−		−	−	
Hemoglobino-philic	33	−	−			−					−	−		−	−	−	−	+	+	−	+	±	±	+
	35	−	±		−	−					−	−		−	−	−		+	+	±	+	±	±	
	37	±			−	−					−	−		−	−	−	−	±	+	+	±	±	−	−
	87	−	−	−	−		−	−	−					−	−	−	−	+	+	±	+	−	−	−
	188	−	−		−	−	−	−	−		−	+	−	−	−	−	−							
	747	−	−	−		−	−	−	−			−		−	−		−				−	+	+	+
	11	−	−		−		−		−			−			−			+	+	±	+	+	+	
	B1$_2$										−			−						±	±	+	+	+
	Z	−		−			−	−	−			−		−		−	−	+	+	+		+	+	+

Serum Strains		Bordet-Gengou															Atypical B.G.					Hemoglobinophilic									
		P.D.	M	55	81	93	95	98	100	110	114	121	141	154	155	P.V.	C.	1	31	10	P.V.	33	35	37	87	188	747	11	B1₂	Z	P.V.
Bordet-Gengou	P.D.	+	+	+	+	+	+	+	+	+		+	+	+	+	+	−	±	±	−	±	−	−	−	−	−	−		−	−	
	M	+	+	+	+	+	+	+		+		+	+	+	+	+	−	−	−	−	±	−	−	−	−	−	−		−	−	
	55	+	+	+			+	+		+	+	+		+	+	+	−	−	±	−	±		−		−	−	−		−		
	81	+		+	+	+	+	+		+	+	+	+	+	+	+	−	−	−	−	±		−		−	−	−		−	−	
	93	+		+	+	+	+	+		+		+	+	±	+		−	−	−	−			−		−	−	−		−	−	
	95	+	+	+	+	+	+	+		+		+	+	+	+	+	−	−	±	−	±	−	−		−		−		−	−	
	98	+	+	+	+	+	+	+		+		+	+		+	+	−	±	±	−	±	−	−		−		−		−	−	
	100	+		+	+	+	+	+	+	+	+	+	+		+	+	−	−	−	−	±	−	−		−		−		−	−	
	110			+			+	+		+		+		+	+																
	114	+	+	+	+	+	+	+			+		+				−	−	−	−					−		−		−		
	121	+		+	+	+	+	+		+	+	+	+		+	+	−	±	−	−	±	−	−		−		−		−	−	
	154	+			+		+	+		+		+	+	+	+	+	−	±		−		−	−							−	
Atypical Bordet-Gengou	C.	−		−	−	−	−	−				−	−			−	+	−	−	−	+	−	−	−	−		−		−	−	
	1	±		±	±	±	±	±		±		±	−	±	±		−	+	±	−	+	−	−	−	−		−		−	−	
	31	+	−	−	+	+		+	+			+			+		−	−	+	±	+				−		−		−	−	
	10	−	+	−	−	−	−	±	±		−		−				−	−	±	+		−		−	−		−		−	−	
Hemoglobinophilic	33	−		−	−											−	−	−	−	−	−	+	+		±	±	±	−	±	−	
	33	−		−						−							−	−	−	−	−	+	+					+	−		
	37				−	±		±				±	±				−	−	−	−			−	+	±	+	−	−	−	−	
	87	−			−			−						−	−		−	−	−	−		±		−	+		−		−	−	
	747	+		+		+		±		+	+	+	±		±	±	−	±	±	−	−	±	±	−	±		+	+	±	±	±
	B1₂	−		−	−			−		−		−				−	−	−	−	−	−	±	±	−	±	±	±	+	+	+	
	Z	+	+	±	+	+	−					+	±				−	±	±	±			−		−		±		±	+	

Tables II and III are composites, made up from the total number of titrations. In almost every instance the results recorded have been confirmed by repeated tests with serums of the same strain from different rabbits and with different antigens of the same strains. + denotes complete inhibition of hemolysis; — denotes incomplete or no inhibition of hemolysis; ± denotes that irregular results have been obtained, either complete inhibition on some occasions and incomplete on others, all tests being apparently equally valid, or that complete inhibition has occurred in a lower dilution than with the homologous strain. The significance appears to be the same in either case, *i. e.,* a relationship of strains not identical. For example, serum P.D. fixed in practically the same amount in a 1 in 100 dilution with four different Bordet-Gengou strains (with 93, 121 and 155 in .0003 cubic centimeter; with 98 in .0004 cubic centimeter); with four different influenza strains and with three atypical Bordet-Gengou strains it gave no complete inhibition in the maximum amount of serum tested, .02 cubic centimeter; with the atypical I it fixed in .003 cubic centimeter, *i. e.,* in a 1 in 10 dilution. Between .0003 cubic centimeter or .0004 cubic centimeter and .003 cubic centimeter there is so great a difference that these two strains, P.D. and I, cannot be considered homologous, though the relationship is rather close. Titrations of Serum I lead to the same conclusion. Antigen titrations of P.D. and I show less difference between the strains but enough to confirm the results of the serum titrations. Other Bordet-Gengou strains have shown a similar relationship to the atypical I and to 31, which stands in about the same relationship to I as to the Bordet-Gengou strains. A similar relationship exists between the Bordet-Gengou strains and the two influenza Strains 747 and z. Serums of Strains 747 and z have fixed with antigens of Bordet-Gengou strains, but no antigen of Strain 747 or z has fixed with a Bordet-Gengou serum, which confirms the experiments of Shiga, Imai and Eguchi.[7] The other influenza strains studied and the atypical C are entirely distinct, according to complement fixation, from the Bordet-Gengou strains studied. The atypical 10 has cross-fixed slightly with Bordet-Gengou strains.

No consistent difference in the Bordet-Gengou strains themselves has been demonstrated, but the subject is still under investigation. Certain influenza strains that appeared to be identical (33-35, BI$_2$-11) were found to be capable of separation if a larger amount of complement was used in the test. For example, No. 107 (strain BI$_2$) when titrated with one unit of complement and one unit of amboceptor fixed in .0003 cubic centimeter with its homologous antigen, in practically the same amount (.0002 cubic centimeter) with Strain 11, in .002 cubic centimeter with Strains 33 and 35, and not even in .02 cubic centimeter with the hemoglobinophilic Strain 37 or the Bordet-Gengou Strain 55. On increasing amboceptor to two units, the results were practically the same. With the use of one and one-half units of complement and only one unit of amboceptor there occurred a corresponding weakening of both strain and group fixation; the antibody unit with the homologous Strain BI$_2$ was .0005 cubic centimeter; with Strain 11, .0004 cubic centimeter; with Strains 33 and 35 the smallest amount that gave complete fixation was .005 cubic centimeter. By increasing the amboceptor to two units a further change in both strain and group fixation was brought about, the unit with BI$_2$ falling to .0009 cubic centimeter, that with 33 to .004 cubic centimeter, and that with 35 to .008 cubic centimeter. With two of complement and one of amboceptor, fixation occurred with BI$_2$ and 11 only, in .0008 cubic centimeter and .001 cubic centimeter respectively. With two units of complement and two of amboceptor, the units were .0009 cubic centimeter with BI_2 and .002 cubic centimeter with 11, which may be identical with BI_2. No tests have been made with more than two units of complement and amboceptor. Similar tests have been made with Bordet-Gengou strains but, even with two units of complement and two of amboceptor, a consistent difference in the strains has not appeared. With a greater increase in one or the other reagent a differentiation may be possible.

Some of the inconsistent results obtained, as in cross titrations of Bordet-Gengou and closely related atypical strains, have undoubtedly been due to variations in the hemolytic system, as an accurate standardization of neither amboceptor nor complement is obtained by titrating amboceptor with ten per cent. complement, as we have done. This crude method of obtaining the balance of the hemolytic system seems, however, to give as uniform results as the use of a definite number of complement and amboceptor units, due probably to variations in the fixability of complement.

Another reason for the lack of uniformity in results arises from the fact that equivalent amounts of antigen or serum were not always used. About two units of antigen, according to a recent standardization with two units of homologous immune serum, were used in serum titrations and about two units of serum in antigen titrations; but as antigen gradually deteriorates and as the complement varies in fixability the antigen unit may change from one day to the next. Unfortunately, it is not always practicable to titrate all antigens before beginning a day's work to assure the use of equivalent amounts of antigen in all serum titrations. By combining the results of a large number of titrations and excluding all that appear questionable we feel that valid conclusions may be drawn from the work.

Conclusions

1. The separation by morphological and cultural characteristics of the typical Bordet-Gengou bacillus from atypical strains of the Bordet-Gengou bacillus and from the influenza bacillus has been confirmed by complement fixation tests.
2. B. pertussis is somewhat related in its complement fixation characteristics to at least two strains of the influenza bacillus and to at least two atypical pertussis strains, but is differentiable.
3. Immune serums of Bordet-Gengou strains have been found to cross-fix with antigens of Bordet-Gengou strains only. Immune serums of some influenza strains have cross-fixed with Bordet-Gengou as well as with influenza strains.
4. Twelve strains of B. pertussis isolated in this laboratory from cases of pertussis have given complement fixation reactions similar to each other and similar to the two strains obtained from other laboratories and may be considered practically homologous.
5. By the use of more highly specific antigens or larger amounts of complement and hemolytic amboceptor than those ordinarily employed a differentiation of individual strains of B. pertussis may be possible.
6. To obtain an immune serum of high complement-fixing-antibody content, four intraperitoneal inoculations of a living culture of B. pertussis or B. influenzæ may be sufficient, nine or more inoculations are advisable, as a decided rise in the antibody content curve occurs after nine inoculations.

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AGGLUTINATION IN PERTUSSIS, ITS CHARACTERISTICS AND ITS COMPARATIVE VALUE IN CLINICAL DIAGNOSIS, AND IN DETERMINATON OF GENUS AND SPECIES.

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Introduction

The work herewith reported is part of a series of studies on pertussis begun by the Bureau of Laboratories of the New York City Health Department in 1914[1], under the direction of Dr. A. W. Williams. The subject of agglutination was taken up chiefly because of the different reports given by various authors as to the value of agglutination and of complement fixation in the early clinical diagnosis of pertussis and because of the comparatively few definite reports made on the value of former test.

Critical Statement of Previous Reports

All authors (at least those who give some details about their work) are agreed that agglutinins for B. pertussis may be produced in most test animals, but statements are vague as to the time and amounts of production and the conditions under which they are produced.

The general statement is usually made that after several inoculations made preferably into the peritoneal cavity, the serum of the animal showed agglutinins. The reports on the strength of the serum, if stated at all, are varied. Thus Bordet and Gengou[2] obtained in rabbits after four inoculations intraperitoneally a serum which the authors say was not as strong as that of the horse which was inoculated many more times subcutaneously and intravenously. Arnheim[5] obtained a serum in rabbits of 1-5000 strength and in the horse 1-10,000, but he gave no details of methods and times of inoculation. Wollstein[7] by intraperitoneal inoculations obtained a serum in the rabbit up to 1-800. Intravenous inoculations were not successful. No details of amounts or time of inoculation were given. Bücher and Menshikoff[9], Shiga, Imai, Euguchi[10] found agglutination tests in pertussis of no value, as their bacillary emulsions were self-agglutinating.

Wollstein[7], Frankel[8], Seiffert,[6] Arnheim,[5] found agglutination tests of some value in clinical diagnosis of pertussis. Renaux[11] did not think it worth while to try.

Bordet[4] finds it of value only under certain conditions, namely, the organism must be freshly isolated from human sputa and grown on rich blood medium. The work of Bordet on agglutination will be dwelt upon more in detail later.

Original Work

Our studies may be divided into:

1. The determination of the best conditions under which agglutinating serum may be obtained in test animals.
2. The comparative value of the test in demonstrating specificity of strains isolated by us from human beings suffering from whooping cough.
3. To add further evidence as to the comparative value of complement fixation and agglutination in the clinical diagnosis of pertussis.

I. *The Production of Agglutinins.*—Rabbits, horses, goats and sheep have been used by us in producing agglutinins. Rabbits and horses responded more readily than the goats and sheep, so most of the tests have been made with serums from the former animals. Practically all of the rabbits inoculated (over 95 per cent.) which have remained in good health have produced abundant agglutinins for *B. pertussis,* though not all equally abundant.

Antigens used.—We have worked with 27 strains of culturally and morphologically typical Bordet-Gengou bacilli (B. pertussis), with 9 strains of B. influenzæ, with 4 strains of B. bronchosepticus, and with 4 strains of bacilli morphologically similar to B. pertussis, but with cultural differences. These last strains we call pertussis-like bacilli. Living emulsions were used for the regular inoculations. In the rabbit, antigen *extracts* similar to those used in the complement fixation test were also tested as well as were regular vaccines and sensitized vaccines for their power to produce agglutinins.

Living cultures of B. pertussis for inoculations were prepared by *scraping* off a 48-hour culture grown on tube slant on Bordet-Gengou medium in recently isolated cultures, and on coagulated horse-blood-glycerine-veal-agar in older strains. The scraped-off culture was emulsified in normal salt solution and the dose graduated according to age of the culture from isolation and to the kind of culture medium used.

The highest virulence for guinea pigs has been ½ slant of a profuse culture inoculated intraperitoneally. This initial virulence on the blood-veal-agar medium became less after the culture had been grown in successive transplants on this medium for a year. Then 1 to 2 slants on coagulated horse blood agar were required to kill a guinea pig of about 200 grms. inoculated intraperitoneally.

The living cultures of *influenza bacilli* were grown for 48 hours on coagulated horse blood agar and then scraped off and emulsified in normal salt solution.

The initial dose of *B. pertussis and of B. influenzae* inoculated intraperitoneally into rabbits was from ⅛ to ½ slant. The last dose was 5-6 cultures on 100 cubic centimeter Blake bottles.

The *pertussis-like* strains were all grown on plain veal agar, on which medium they grow abundantly. The emulsions were made in the same way as the others. These strains were very virulent for rabbits; one especially, No. 31, which had to be given at first in doses of 1/100 to 1/50 of a slant. The last dose was four slants.

The *antigen extracts* were prepared in the same manner as were those for complement fixation tests, namely, a 48-hour growth of B. pertussis on coagulated horse-blood-veal-agar medium was scraped off in distilled water shaken for two successive days brought up in the water bath to 56° and left in the incubator at the same temperature over night; the next day the emulsion was centrifuged and the supernatant fluid, rendered isotonic by adding 9 per cent. of salt, was injected into the animals.

The dosage of this antigen was ¼ c. c. intravenously, ½ c. c. intraperitoneally or 1 c. c. subcutaneously. The dose was gradually increased up to 10 c. c. The toxicity of this antigen was tested and it was found that 5-10 c. c. of it killed guinea pigs of 200 grams in weight, inoculated intraperitoneally in 24 hours; 3 c. c. had no effect.

The *vaccines* were made by scraping a 48-hour growth of B. pertussis on coagulated horse-blood-veal-agar, emulsifying in physiologic salt solution, shaking the emulsion for two successive days and then heating it at 56° for one and one-half hours. The initial dosage of the vaccine which was usually 15 billions per cubic centimeter strong was ¼ c. c. intravenously, ½ c. c. intraperitoneally or 1 c. c. subcutaneously. Its toxicitv was such that 10 c. c. of a 15-billion-strong vaccine killed guinea pigs of about 200 grams weight inoculated intraperitoneally in 24 hours. Three to 5 c. c. had no effect.

The *sensitized vaccines* were prepared the same way as the killed vaccines, except that they were shaken only one day and instead of being heated were agglutinated by 1-100 immune pertussis serum for one hour in the incubator at 37° and were then placed in the ice box over night. The next day the precipitated emulsion was shaken up (the supernatant fluid is quite clear) and washed two to three times by physiological salt solution. The dosage of this vaccine was the same as of the killed one, but it was usually 10 instead of 15 billions per c. c. strong. Its lethal dosage is still being tested in order to gain more explicit data about the comparative virulence of living and sensitized pertussis organisms. Thus far we found that 1:40 immune rabbit pertussis serum added to 3 c. c. of a 10-billion per c. c. strong vaccine prevents the death of 200 gram guinea pigs, while the control mixtures of normal rabbit serum added to the same amount of emulsion killed the pigs by the next day. The emulsions of non-sensitized bacilli in one cubic centimeter doses killed the pigs in 24 hours. The sensitized vaccine organisms remain alive in the ice box for 2-3 weeks, when the growth gradually decreases; on the fifth week only a few colonies are obtained. The untreated organism lives in the ice box about two months. More work is being done on the bacterial toxicity of these immune serums.

Technique of agglutination tests.—It is of vast importance to obtain homogenous emulsions, especially for microscopic tests. The importance of the culture medium and the subsequent treatment of the cultures for agglutination tests has thus far never been dwelt upon in detail by any writer on this subject except perhaps Bordet[3] himself and then only on some phases of it. The B. pertussis when recently isolated grows only on Bordet-Gengou medium, but after five to six generations it grows much more abundantly and characteristically on glycerin-veal-agar .4 acid to phenolphthalein to which horse's blood (1-75 to 1-100) has been added while the agar is very hot (90°). The influenza bacillus also grows excellently on this medium, with the exception that the reaction should be neutral to phenolphthalein. Though the pertussis organism grows equally well on the neutral medium, it forms an adherent growth which cannot be separated from the medium and the acid reaction seems to prevent this occurrence. The cultures for the tests are taken from a 48-hour growth on this acid medium and allowed to grow for 24 hours on 1-100 coagulated horse's-blood-veal-agar *neutral* in reaction. A 24-hour growth usually is not adherent on neutral medium in the first generation from the acid medium. The glycerin is omitted for the last growth, as it seems to interfere with the reaction. Recently isolated strains, of course, must be grown on Bordet-Gengou medium. The growth is scraped off, not washed off, in distilled water and shaken in an electrical shaker from three to four hours. A standard emulsion is kept in the ice box, so that the emulsions are always uniform. The sera are diluted with .85 per cent. salt in 1 to 10, 25, 50, 100, 200, 400 parts, etc. Each test tube contains ¼ c. c. of the diluted serum and ¾ c. c. of the emulsion, the final dilution being 1:4. The tests are macroscopic with the animal sera and microscopic with patient's sera unless there is enough serum for the macroscopic test. The test tubes and hanging drops are put in the incubator at 37° from one to two hours and readings made; the macroscopic tests are left over night in the ice box. Complete agglutination is designated by ++; incomplete by +; slight by ±.

With this technique we have not as yet encountered a self-agglutinating strain on coagulated horse-blood-veal-agar medium, and only in the very beginning a few recently isolated strains on Bordet-Gengou medium were slightly self-agglutinating (perhaps they were not shaken long enough).

The glassware must be absolutely clean and antiseptics such as bichloride of mercury avoided. For the examination of patient's sera, one or more strains of B. pertussis of known agglutinability and one influenza strain are used. One recently isolated strain is grown on rich Bordet-Gengou medium (according to the suggestion from Bordet), the other on our standard medium (coagulated horse-blood-veal-agar). The last medium as a rule gives the most satisfactory results, especially for hanging drops, as it invariably forms a homogenous mass of entirely separated organisms, and when a reaction occurs it is quite unmistakable.

First appearance of agglutinins.—Agglutinins, as a rule, did not begin to appear in the rabbit until after the sixth or seventh inoculation, at least in no higher strength than 1:40 or 1:100. After that, if the animal kept well, the agglutinins continued to rise with each inoculation. Most rabbits were sacrificed when they showed from 2400-2500 titre, which happened usually after the tenth to the twelfth inoculation. Some of them might have possibly shown a higher titre if further inoculated.

Normal rabbits show agglutination in dilutions not higher than 1:25; sheep and goats 1:40-1:100; horses up to 100. The sheep and goats seemed to respond more slowly to the inoculations than the rabbits and horses, so they were dropped. Two of the three horses—one by intravenous, the other by subcutaneous inoculation—showed agglutinins up to 2000 after the third and fourth inoculation. Two horses are still in the process of immunization.

The injection of living cultures seems to be most conducive to the development of agglutinins in the rabbit, though now and then there is a rabbit found which shows fairly good agglutinins after four to six inoculations with killed vaccines, sensitized vaccines and antigens, though the titre has never become as high as with living cultures.

Some of the rabbits inoculated with killed vaccines and more of those inoculated with sensitized vaccines and antigens (8-9 inoculations each) did not develop agglutinins to any extent, though they were markedly immune. Thus, five of such rabbits were injected with four to five surface growths of 100 c. c. bottles without ill effects, while the control rabbits died from the same dose or less (three bottles) the same day or next morning from acute toxemia. Two rabbits more responsive to the vaccines developed after a few inoculations a comparatively high agglutination titre up to 1000. The low-titre rabbits were inoculated later with living cultures, and after a few inoculations showed a high agglutinating power. The influenza rabbits did not form agglutinins as readily as did the pertussis rabbits and the titre never reached higher than 1 to 1600. The following table shows the comparative rise of agglutinins and of complement fixing bodies in rabbits inoculated with B. pertussis, B. influenza and pertussis-like bacilli.

II. *Specificity of strains from whooping cough cases.*—From table II, which shows the agglutinative reaction of pertussis sera with heterogenous strains and attempts at cross-agglutination with *B. influenza,* and pertussis-like bacilli, it would seem that not only do the pertussis strains show no relationship to the pertussis-like strains or to the influenza strains, but that all strains of typical pertussis bacilli belong to one group. This is in marked contrast to the reaction of influenza sera which agglutinate most heterogenous strains in very low dilutions or not at all. The pertussis-like organisms also show a variation in strains. Thus of the four strains tried, No. 31 and No. 1 seem to be identical. The other seem to be quite distinct strains. In no instance is there cross-agglutination of B. pertussis serum with B. in-

TABLE I

COMPARATIVE RISE OF AGGLUTININS AND COMPLEMENT FIXATION ANTIBODIES, WITH SUCCESSIVE INOCULATIONS OF B. PERTUSSIS, B. INFLUENZA, AND PERTUSSIS-LIKE BACILLUS BY INTRAPERITONEAL INJECTIONS (a)

Initial Weight Grams	Last Weight Grams	Initial Dose	Last Dose	Strain	After 3d Inoc.		After 4th Inoc.		After 5th Inoc.		After 6th Inoc.		After 7th Inoc.		After 8th Inoc.		After 9th Inoc.		After 10th Inoc.		After 11th Inoc.		After 12th Inoc.		After 13th Inoc.		After 14th Inoc.		After 15th Inoc.	
					Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.
															Pertussis Rabbits															
1,920	1,840	½ slant	3 slants	P. D	*	001	1:40	0003	Rabbit sacrificed after 4 inoculations																					
2,100	2,070	¼ slant	Surface growth of 3 100 c.c. bottles	55	0	0	0	0	0	005	*	003	1,200	002	*	002	1,000	001	2,400 no more dilutions tried	001	5,200	Rabbit sacrificed								
1,420	1,780	⅛ slant	Surface growth of 4-100 c.c. bottles	93	0	005	0	004	0	003	0	001	*	0009	2,400	001	5,200	Rabbit sacrificed												
1,380	1,750	¼ slant	As above, 3 bottles	110	0	004	0	0	0	005	*	0004	400	0007	1,200	0004	1,600	Rabbit sacrificed												
2,040	1,880	1 slant on plain veal agar	As above, 12 bottles	P. D	0	0	0	0	40	0	1,200	001	2,000	004	2,400	0005	2,000	002	2,000	0004	2,100	008	2,400	0008	3,200	002				
1,315	1,565	¼ slant	As above 4 bottles	154	0	0	0	0	0	0	0	0	0	0	0	001	*	001	800	004	1,200	0	1,000	0	800	0				
1,870	2,300	½ slant	As above 9 bottles	55 93	0	0	0	0	200	0	1,000	001	2,000	005	*	0008	2,400	001	2,400	0003	4,000	0003	10,000	0003	sacrificed					
1,700	2,130	Sensitized vaccine, ¼ c.c.	Living culture, surface growth 6 100 c.c. bottles	154 155 163	0	0	0	0	40	0	*	02	200	002	*	003	400	001	2,000†	002	2,100	002	2,400	002	*	002	4,000	001		
1,950	2,110	Extract "antigen" 1 c.c.¶	Living culture, surface growth 1-100 c.c. bottles	154 155 163	0	0	0	0	0	0	0	0	0	0	0	0	40	003	100	0	*	006	200†	0	1,200	008	2,100	*	6,000	001
2,670	2,730	Killed vaccine ¼ c.c.	8 c.c.	154 155 163	0	003	0	*	400	002	1,600	0005																		
															Influenza Rabbits															
1,910	2,350	¼ slant	Surface growth of 8 100 c.c. bottles	37	0	0	0	0	0	0	0	0	0	0	0	0	0	006	0	0	200	001	40	003	400	test unsatisfactory				
1,430	1,660	½ slant	As above 5 100 c.c. bottles	B12	0	0	0	0	0	0	0	003	0	003	0	001	40	0007	0	003	200	001	200	002	200	0003	40	0005		
1,310	2,080	¼ slant	As above 8 100 c.c. bottles	35	0	0	0	0	0	004	0	003	0	004	0	002	0	0009	200	0003	*	*	0	0009						
2,220	2,300	⅛ slant	As above 11 100 c.c. bottles	747	0	0	0	0	0	0	0	0	0	003	200	0008	200	0	*	*	40	0	200	006	200	006	10	*	200‡	005
											Rabbits inoculated with pertussis-like bacillus																			
1,905	1,710	1/50 slant	3½ slants	31	0	004	0	008	0	005	0	009	*	009	1,200	009	2,200	001												
1,980	2,365	⅛ slant	6 slants	C.	0	0	0	005	0	0	0	006	0	007	0	005	0	003	0	006	0	003								
1,565	1,620	⅛ slant	1 slant	1	*	*	1,200	0006	sacrificed																					

* Not tested

† After injection with living cultures intraperitoneally.

‡ After the 17th inoculation this rabbit reacted up to 1:1,600; after the 18th inoculation, it dropped down to 1:400; at the end of the 21st inoculation the reaction was only 1:200. Complement fixation varied from 0008 to 0001.

¶ Subcutaneously.

(a) Injections with living cultures were given once a week, vaccines and antigens were given twice a week till the 6th inoculation, then once a week.

TABLE II.

Agglutination Tests of Pertussis Serum with Homologous and Heterogenous Strains, B. Influenza, Pertussis-Like Bac. and B. B.

Pertussis Serum	Pertussis Strain	Titre of Agglutination in Incubator at 37° C. for 1½ Hours	40	400	2,000	2,400	2,800	3,200	4,000	5,000	6,000	7,000	8,000	10,000	Control
	55	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
	M.	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
	93	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	95	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
	P. D.	8,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	163	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	155	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	154	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	109	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
	188 R.	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
	100	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	98	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	110	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
	114	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—

TABLE II.—*Continued.*

Pertussis Serum	Influenza Strain	Tried in Dilution 1:40, 100, 200, 400, etc.	40	400	2,000	2,400	2,800	3,200	4,000	5,000	6,000	7,000	8,000	10,000	Control
	747	—													
	37	—													
	33	—													
	35	—													
Pertussis Serum	Pertussis-like Strains														
	31	1:40, 100, 200	+	—	—										
	C.	+ — — 1:40, 100, 200 + — —	+	—	—										
	10	— — —	—	—	—										
	1	+ — —	+	—	—										
Pertussis Serum	Broncho Strains														
	155	40, 100, 200 ± — —	±	—	—	. .	. .	. .	. .	. .	. .	. .	. .	. .	—
	156	± — —	±	—	—	. .	. .	. .	. .	. .	. .	. .	. .	. .	—
	97	++ ± ± Control ±	++	±	±	. .	. .	. .	. .	. .	. .	. .	. .	. .	±

fluenza even in 1:40. The serum of the pertussis-like bacilli and the influenza sera sometimes agglutinate B. pertussis in 1:40, but never higher. Of the four strains of B. bronchosepticus used, none showed any relationship to pertussis agglutinins. The variability of hemoglobinophilic strains has been shown by previous work[12].

Absorption tests verified the supposition gained from the straight agglutination tests that all pertussis strains belong to one species as far as agglutination reactions are concerned. In order to do absorbtion tests, the serum must be saturated by a very thick emulsion, as the least agglutinin not absorbed may be shown markedly by sensitive strains more agglutinable than others, and in this way obscure the test. Thus one rabbit was inoculated with three strains which we thought might be a little different from each other on account of their varied powers to agglutinate in weak serums, but by absorption tests they proved to have identical agglutinins for themselves as well as for all the other strains[4] tested.

Variations in agglutinability of pertussis strains.—Though all pertussis strains are practically equally agglutinated by a strong serum, individual strains vary in regard to promptness of response, and with a weak serum heterogenous strains may show marked difference in the quantity of serum required for a reaction. We have often seen the phenomenon observed by Bordet[2] and others of the agglutination of heterogenous strains more strongly than the strain with which the antiserum was obtained. This characteristic of B. pertussis is brought out more clearly by weak serums. Thus some of our serums from rabbits and goats after a few inoculations hardly agglutinated their homologous strains while they agglutinated a heterogenous strain up to 1 to 2000. It is important, therefore, in testing patients' serums to use a strain of known agglutinability (as in typhoid).

The difference in the agglutinability of different pertussis strains was well brought out in the instance of one of our rabbits inoculated with three strains, M, 55 and 93. After five inoculations its serum agglutinated strains 93 and M, and the heterogenous strain P.D. up to 200, but not at all strain 55. After six inoculations M, 93 and P.D. were agglutinated up to 2000 and 55 only very slightly in the first few dilutions. After the ninth inoculation 55 was agglutinated up to 200, while the other strains showed very strong agglutination up to 2400. After the tenth inoculation all four strains (M, 55, 93, P.D.) showed very strong agglutination up to 2400 (no more dilutions were tried.) After eleven inoculations all strains were agglutinated up to 4000 (no higher dilutions tried). After the twelfth inoculation agglutinins for all three homologous strains and for twelve other strains tried were 1 to 10,000 in a trial serum. It would seem that only a very strong serum could overcome the sluggishness of strain 55. Another strain of this kind is No. 163. Rabbits inoculated with 155, 154 and 163 showed very high agglutination with both strains 154 and 155 before 163 was agglutinated.

Another interesting feature in the agglutinations of B. pertussis is that when a mixture of strains is used in making the emulsions for agglutination the reaction occurs more promptly than when each strain is used alone. Why this occurs we do not yet know. Because of it we are making comparative tests with a mixture of three or more pertussis strains in emulsion and a one strain emulsion for testing patient's sera.

According to Bordet[4] the age of a culture from date of isolation influences the agglutinating property of *B. pertussis*. He cites an experiment in which he tested a pertussis serum stored in the ice box for four years with its homologous strain kept transplanted all these years on Bordet-

Gengou medium. No agglutination occurred, though this strain was highly agglutinated by the same serum four years ago. The natural conclusion at first was that the serum had lost its potency, but when it was tested with a freshly isolated strain it gave a strong agglutinating reaction. Bordet, therefore, advocates for testing patient's sera for agglutination to use freshly isolated cultures grown on rich blood medium.

In our experience we have found that while freshly isolated strains on Bordet-Gengou medium are quite responsive to a strong serum the most readily agglutinable strains are a few that were isolated from one to one and one-half years ago and which grow best at present on our standard coagulated horse-blood-veal-agar medium. Of course we cannot predict how modified these strains may be in four years.

Relationship of culture media to agglutination.—Bordet[3] states that animals inoculated with *B. pertussis* grown on Bordet-Gengou medium develop agglutinins for *B. pertussis* grown only on the same medium, not for the organism grown on plain agar and vice versa. He also states that by absorption tests he found that these two agglutinins were not the same.

In testing these experiments we worked with three media; Bordet-Gengou, coagulated horse-blood-veal-agar, and plain veal-agar (only two of our strains grow well on plain agar thus far). When working with weak sera (sera which do not give a prompt reaction in the incubator at one and one-half hours, though they may agglutinate up to 2400 the next morning) and freshly isolated strains, we were impressed by the truth of Bordet's statements. Thus horse I, injected three times with two strains grown on Bordet-Gengou medium did not agglutinate the same strains grown on coagulated horse-blood-veal-agar, but agglutinated these very strains up to 2000 when they were grown on Bordet-Gengou medium, but the same serum agglutinated our older agglutinable strain grown on coagulated horse-blood-veal-agar in just as high dilutions. On the other hand a rabbit inoculated with a well agglutinable strain grown on plain veal-agar after six to seven inoculations agglutinated up to 2000 equally well, the same strain grown on each of the three media. Absorption tests did not seem to bear out the statement that the agglutinins were different. From our experience it would seem that the culture medium influences an agglutinable strain in so far as it affects its growth and best development and not in its production of different kinds of agglutinins. Recent strains grow well only on Bordet-Gengou medium and are, therefore, more agglutinable on this medium than when grown on coagulated horse-blood-veal-agar on which they grow in the beginning very scantily. The sluggish strains which grow well on either medium seem to be influenced by the culture media when tested with weak sera. Thus the serum of the same horse injcted with cultures on Bordet-Gengou medium which agglutinated an easily agglutinable old strain on coaglulated horse-blood-veal-agar did not agglutinate a sluggish strain on the same medium (though it grows on it profusely), but agglutinated it when it was grown on Bordet-Gengou medium.

A strong serum, therefore, in our experience agglutinates equally well, though maybe not equally promptly, all strains, sensitive and sluggish, no matter on what medium grown.

Comparative value of complement fixation and agglutination tests in the differentiation of strains.—Agglutinins as a rule (as mentioned before) did not begin to show in the rabbit in any marked degree till after the sixth or seventh inoculation, while complement fixation antibodies as seen from the accompanying table usually appeared after the fourth inoculation, but also showed a higher titre after the eighth to ninth inoculation. Though complement fixation and agglutination outside of the time limit ran quite parallel, namely, most of the animals which show high complement titre show also strong agglutinins, there were rabbits which showed high complement titre and no agglutination and at times vice versa. Especially was this the case when the rabbit showed a very high complement titre after a few inoculations. Thus rabbit No. 28 inoculated with *B. pertussis* after the fourth inoculation showed a complement titre of .0003 and agglutination only 1:40. On the other hand rabbit No. 92 showed complement titre of .001, while it agglutinated up to 5200. Many influenza rabbits showed a high complement titre and very little or no agglutination. On the whole, however, agglutinins in pertussis rabbits, though produced more slowly, were produced more regularly and more surely than were complement-fixing substances.

From our observations we conclude that the agglutination of B. pertussis by artificially produced immune serum gives a more clear-cut differentiation from pertussis-like bacilli and from hemoglobinophilic bacilli than does complement fixation which shows a cross-fixation in low dilutions with some of these latter organisms (Olmstead and Povitzky)[14].

III. *The comparative value of agglutination and complement fixation in the clinical diagnosis of pertussis.*—Sera from 59 cases of pertussis in various stages of the disease were tested for agglutinins. These results, also the results of control sera of adults suffering from different diseases and of children with scarlet fever are given in Table III.

We see by this table that we cannot depend upon agglutination as a positive guide to diagnosis in dilutions up to 1 to 100. If, however, the reaction at 1 to 100 is very prompt (in an hour at 37° C.) and complete, it may be considered suspicious since in none of the controls did the reaction recorded occur quickly. When a reaction occurs in dilutions of 1 to 200 it indicates a probable recent infection with B. pertussis.

Unfortunately, we did not always get enough serum from patients to test both agglutination and complement fixation, so our comparative table is not of the same cases, but the series of those tested for complement fixation is taken from those quoted in the paper of Olmstead and Luttinger[13] that did not receive vaccines. According to this tabulation a positive specific complement fixation did not occur quite as frequently in the first week as did agglutination at 1 to 200. On the other hand, out of 51 control sera of adults only two showed a positive reaction by complement fixation, while about 33 per cent. showed a reaction up to 1:40 by agglutination tests and about 17 per cent. showed a reaction 1:100. Control children sera showed in 40 per cent a reaction in 1:40.

Only three out of the 59 pertussis sera showed agglutination with B. influenzae, two in 1:40 and one in 1:200 at the first week of whoop.

The control sera showed agglutination with B. influenza only in two cases in 1:40, while complement fixation showed fixation with influenza quite frequently.

SUMMARY

1. A strongly agglutinating pertussis serum was best obtained in the rabbit by 10-12 intraperitoneal inoculation of living cultures given at seven-day intervals. Over 95 per cent. of rabbits, inoculated in this manner, produced agglutinins, though not in equal abundance. Agglutinins are also produced by killed vaccines, but not as abundantly as by living cultures. Those rabbits which received eight-nine inoculations of vaccine and did

TABLE III

Patients' Pertussis Sera

Agglutination Reactions						Complement Fixation Tests					
Weeks Whoop	No. of Cases	—	1:40	1:100	1:200	Weeks Whoop	No. of Cases	—	+	++	+++
1	13	5	4		4	1	6	4	0	1	0
2	10	0	6	3	1	2	4	3	0	0	1
3	9	1	0	6	2	3	5	0	2	2	1
4	13	2	4	6	1	4	2	0	1	0	1
5	2	0	0	0	1	5	4	1	0	1	2
6	4	0	4	0	0	7	2	2	0	0	0
14	1	0	0	1	0	17	1	0	0	0	1
20	1	1	0	0	0	Convalescent 8	1				1
Convalescent 1	2	1	0	1		Convalescent 5 mos	1				1
Convalescent 8	1	1									
Convalescent 5 yrs	1	1									
Exposed to W. C. 2 weeks	2		2								
					Control Sera Adults						
	58	29	19	10			51	49	2		
				Control Sera Children (Scarlet Fever)							
	10	6	4								

not show agglutinins to any large extent were, nevertheless, markedly immune.

A high-titre serum (from 1:4000 to 1:10,000) always proved to be a quickly acting serum, reacting in from one to one and one-half hours in the incubator at 37°, sometimes up to 1:8000 with well agglutinable pertussis strains.

A low-titre serum was usually slow in reaction and did not show agglutination till after remaining in the ice box over night.

2. By agglutination tests B. pertussis strains can be specifically identified from hemoglobinophilic baccili, pertussis-like bacilli and bacillus bronchisepticus. In no instance was there cross-agglutination between these organisms, at least not higher than 1:40. For identification a strong serum of high titre should be used.

3. By these tests it would also seem that all pertussis strains isolated in our laboratory belong to one group. A strong serum agglutinated all our pertussis strains (27 in number) equally well, though not equally promptly in the highest dilutions. Absorption tests (tried with seven most variable strains) proved the supposition gained by the straight agglutination tests.

4. The variation of the agglutinability of pertussis strains with weaker sera depends very much on the age of the culture (dating from isolation) and culture medium. Freshly isolated strains are best agglutinable on Bordet-Gengou medium; older strains on our standard coagulated blood-veal-agar.

5. A strong serum agglutinates equally well, though not equally promptly all strains, no matter on what medium and how sluggish the strain is.

6. Agglutination tests in clinical diagnosis of pertussis compare favorably with complement fixation only in the first week of the whoop. In the later stages of the disease complement fixation antibodies appear more frequently than agglutinins.

7. About 33 per cent. of adult non-pertussis sera showed agglutination in up to 1:40; 17 per cent. in up to 100. Forty per cent. of children's non-pertussis sera (scarlet fever cases) showed a reaction in up to 1:40.

A dilution of not less than 1-200 is necessary for a practically positive diagnosis of pertussis by agglutination test.

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THE RELATIVE VALUE OF CERTAIN METHODS FOR THE PRODUCTION OF ANTI-SHEEP AMBOCEPTOR

Rose Schweitzer and Valentine Stevens

The value of any method for producing haemolysins depends upon two factors: a low percentage of mortality and the potency of the serum obtained. We have investigated five different methods and believe to have developed one for obtaining anti-sheep amboceptor which provides for both these factors.

The following are the methods tested: *Method No. I.* This is a method suggested by Kolmer[1] and consists of weekly inoculations of 3, 5, 15 and 20 c. c. of a ten per cent. suspension of sheeps' cells; the animals being bled four days after the last inoculation. We were obliged to abandon this method because our animals invariably died after the second inoculation. We also tried another of Kolmer's methods, *i. e.,* that of giving three inoculations of 5 c. c. each of a ten per cent. suspension, every third day. We found, however, that the final titre of the serum was only as high as that which we obtained with the method No. *V* after the third inoculation.

Method No. II. This was first suggested by Gay and Fitzgerald[2] and consists of one c. c. inoculation of a fifty per cent. suspension on three successive days. We used seven rabbits in this series (almost as many as the authors worked with). However, our results were not as satisfactory as those reported by the authors.

Methods III, IV, and V embrace our own experiments. In *Method No. III* we gave intravenous injections of 1, 3 and 5 c. c. of a fifty per cent. suspension of erythrocytes on three successive days. In *Method No. IV* the dosage was changed to 2, 4 and 6 c. c. and inoculations were made on every third day. The rabbits were bled from seven to nine days after the last inoculation. Thirteen rabbits were inoculated with method No. *III.* Six died giving a mortality of 46 per cent. The highest unit obtained with this method was .00003 c. c., the lowest unit was .0005 c. c. and the medium unit was .00016 c. c. Seventy-one rabbits were inoculated with method No. *IV.* Thirty-three died giving a mortality of 46.5 per cent. The highest unit obtained with this method was .00002 c. c. The lowest unit obtained was .0007 c. c. and the medium was .00015 c. c. It is evident that both these methods give serum of a high titre. The high percentage of mortality, however, makes them impractical for general laboratory use.

The fifth method was carried out as follows: Inoculations began with ¼ c. c. of a fifty per cent. suspension and increased with ¼ c. c. every third day, giving from four to eight inoculations. The final bleeding took place from seven to nine days after the last inoculation. Small quantities of blood were obtained previous to each injection, and the serum tested to determine its potency. We found it advisable to give the inoculations very slowly.

We consider four or five inoculations to be sufficient, although a satisfactory potency can often be obtained with only three inoculations. We found after the third inoculation haemolysins appeared in amounts of .00004 to .00005. Comparing these units with the medium units obtained with methods *III* and *IV,* we find that the same degree of immunization is reached with this method after the third inoculation as in the other two after the final bleeding.

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[1] Kolmer's "Infection, Immunity and Specific Therapy," p. 72

[2] University of California Publications in Pathology, "An Improved Rapid Method of Producing Precipitins and Hemolysins," Frederic P. Gay and T. G. Fitzgerald, p. 79.

Rabbits that received eight inoculations showed a lower titre after the final bleeding than after the last inoculation. And rabbits that received six inoculations retained the same titre after the final bleeding as after the last inoculation.

Eighteen rabbits were used in this series. One died giving a mortality of 5.5 per cent. The highest unit obtained with this method was .000018 c. c. The lowest unit .00007 c. c. and the medium unit was .00004.

To facilitate bleedings from the ear vein and inoculations, the animals were placed in a Cornell animal box. The final bleedings were effected in a sterile manner, the blood being collected in a large tube by means of a canula and left at room temperature until clotted, then placed in the ice box over night. The immune serum was drawn off with sterile precautions on two successive days and put up in volumes of two cubic centimeters, heated for thirty minutes at 56° C. and kept in the ice box at 0° C. Serum preserved in this manner retains its titre unchanged for months.

The following system was used in determining the titre of the sera: Titrations were started with one in a hundred dilutions, as an amboceptor whose titre was less than one in a hundred was not considered usable. .1, .09, .08, .07, .06, .05, .04, .03, .02, .01 c. c. of the diluted amboceptor were used, .1 c. c. of 10 per cent. pooled guinea-pig complement, 1 c. c. of five per cent. suspension of erythrocytes and the whole balanced with 0.85 per cent. saline solution to make a final volume of .5 c. c. in each tube. The titrations were incubated for one hour in the water bath at 37° C. The sera were titrated in several dilutions until the smallest amount that gave complete haemolysis, that is the unit,* was obtained.

According to our finding it is best to make the final bleeding within four or five days after the last inoculation. Of ten rabbits bled daily for nine days after the last inoculation, a slight or decided drop in the titre was observed after the fifth or sixth day.

The presence of natural anti-sheep amboceptor does not seem to have any effect upon the potency of the serum. The titre as it appears in trial bleedings in rabbits that possessed natural anti-sheep amboceptor was not higher than that of those which showed no natural amboceptor.

Methods III and *IV* produce sera of satisfactory potency but give also a very high percentage of mortality. *Method No. V* produces serum of a higher titre and gives a much lower percentage of mortality. The lower mortality might be due to the fact that the quantities inoculated are comparatively smaller than that of the other methods. Kolmer's and Gay and Fitzgerald's methods give not only a high per cent. of mortality, but also produce serum of a lower titre than our method No. *V*.

CONCLUSION

Of the five methods for producing anti-sheep amboceptor investigated by us our method No. *V* seems most preferable since it results in the lowest percentage of mortality and in the most potent immunine serum.

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*NOTE.—As 1 c. c. of ten per cent. complement is rarely an excess, the amboceptor unit was not the actual unit, according to Bordet's definition, but it was a measure of the value of amboceptor for Wassermann work. In order to obtain a more accurate determination of the comparative potency of sera tested on different days it is of course necessary to use an excess of complement. To insure this we now use .2 or .3 c. c. of ten per cent. complement depending on the complement titre. The sera tested since the adoption of the latter technique are not included in the following or on the observations based thereon.

Method of Immunization	No. of Rabbits Inoculated	No. of Rabbits Died	Percentage of Mortailty	Potency of Serum After Three Inoculations		
				Highest Unit	Lowest Unit	Medium Unit
Method No. II Gay and Fitzgerald's 1cc of 50 per cent. suspension on three successive days......	7	1	14	.00003 cc	.0005 cc	.00016 cc
Method No. III Inoculations of 1-3-5 cc of 50 per cent suspension on three successive days...........	13	6	46	.00003 cc	.0005 cc	.00016 cc
Method No. IV Inoculations of 2-4-6 cc of 50 per cent. suspension every third day. .	71	33	46.5	.00002 cc	.0007 cc	.00015 cc
Method No. V Inoculations of 50 per cent. suspension in series of increasing doses beginning with a ¼ cc and increasing with a ¼ cc every third day..........	18	1	5.5	.000018	.00007	.00004

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COLLECTED STUDIES

From the

Bureau of Laboratories
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Dr. WILLIAM H. PARK, Director

VOLUME IX
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DEPARTMENT OF HEALTH
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DEPARTMENT OF HEALTH

OF THE

CITY OF NEW YORK

BOARD OF HEALTH, 1916–1919

Commissioner and President of the Board
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ROYAL S. COPELAND, M. D., from 1918.

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LELAND E. COFER, M. D., from April 20th, 1916.

Commissioner of Police.
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Hon. RICHARD E. ENRIGHT, from 1918.

EDITOR'S NOTE

Owing to the pressure of work incident to the war no volume of Collected Studies has been issued from this laboratory since 1915. During the interval the majority of the articles in the present volume have been published elsewhere and with some revision are reprinted here from the various medical journals in which they originally appeared.

No papers have been included in the present volume which were written later than the end of 1919. In three instances, however, additions have been made to the original papers in order that the record of observations may be brought up to date. These are: "Epidemic Meningitis," page 13; "Active Immunization Against Diphtheria in a Large Child-Caring Institution," page 354, and "Active Immunization of Infants Against Diphtheria," page 364.

In certain cases, for the sake of brevity and more systematic arrangement, several papers on the same topic have been combined to form one article.

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FRANK SIDNEY FIELDER, Ph. B., M. D.

Since the publication of our last volume of Collected Studies, of which he was the editor, the Bureau of Laboratories has suffered a serious loss in the death of Dr. Frank Sidney Fielder, who died of pneumonia January 18, 1917, after an illness of only six days.

During a period of ten years, Dr. Fielder was in charge of the work of the Vaccine Division, and for half this time the Rabies Division was also under his direction. While in this responsible position, he introduced many improvements in the methods for the production of vaccines.

EPIDEMIC MENINGITIS

Clinical and Laboratory Studies.*

JOSEPHINE B. NEAL.

Introduction
Epidemiology
Etiology
Incubation
Clinical Description and Classification
Meningism
Examination Spinal Fluid
Differential Diagnosis
Treatment
Serum Reactions
Complications and Sequelae
Case Histories
Meningitis in Children Under One Year
Prognosis and Mortality

INTRODUCTION.

The observations embodied in the present article are given as the result of experience gained in the service of the Meningitis Division of the Bureau of Laboratories of the New York City Department of Health. The Meningitis Division was established by Dr. Park in July, 1910, but it is probable that many physicians are still unaware of its existence and of the work it carries on.

As all forms of meningeal involvement are comparatively rare at ordinary times, it is highly desirable to have a force of technically trained and experienced physicians to cope with such diseases. This is especially true of a large city like New York where in the epidemics of meningitis and poliomyelitis which occur from time to time medical resources are sometimes taxed to the utmost. At all times it is to the advantage of the general pracitioner to have within reach specialists upon whom he can always call for assistance in the diagnosis and treatment of meningeal conditions. While the administration of diphtheria antitoxin, for instance, is a rather simple matter with a fairly well established and constant dosage, the technic of lumbar puncture and the administration of anti-meningitis serum is much more complicated. The outcome of the case depends in no small degree upon the experience of the physician in dealing with meningitis.

The work of the Meningitis Division is arranged as follows: All cases of suspected meningitis or poliomyelitis reported to the borough offices are referred to this Division, whereupon we call up the attending physician and offer to see the patient with him if he so desires.

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* A combination of six articles published in:
Jour. Am. Med. Ass., 1916, 66, p. 862.
Arch. Pediatrics, 1917, 34, p. 561.
Clinics North America, 1918, 2, No. 1.
Internatl. Clinics, 1918, 4, Series 28.
Monthly Bull, Health Dept. City of New York, 1918, 8, No. 9.
Long Island Med. Jour., 1920, 14, p. 229.

We see a patient only in company with the physician in charge of the case and at his request for a number of reasons. First, we wish to work with the private practitioner, not to encroach in any way upon his practice. Of course a diagnosis can be more satisfactorily reached in consultation and moreover the treatment of cases of epidemic meningitis requires the closest possible cooperation. Secondly, doing a lumbar puncture and administering serum with necessary antiseptic precautions usually require more assistance than the family can give, and the presence of their own doctor makes them more ready to submit to a procedure which is to them more or less terrifying. Thirdly, when the patient is very ill and dies a few hours after the puncture, the family are better satisfied if their own doctor has agreed with us as to the advisability of the operation.

Since the establishment of the Division, consultations have been held in 2.918 cases. These have included besides epidemic meningitis, tuberculous meningitis, purulent meningitis of all kinds (pneumococcic, streptococcic, influenzal and staphylococcic), poliomyelitis, encephalitis and many rarer conditions such as brain abscess, brain tumor, meningeal hemorrhage, syringomyelia and syphilitic affections of the central nervous system. Of these last we see but few. We are also called upon in many cases of meningism developing in connection with pneumonia and other acute infections especially in children.*

---

* Considerable time may be saved if physicians, wishing us to see a patient, call up the meningitis division, located at the Research Laboratory of the Department of Health. This does not take the place of the report to the borough office. Spinal fluids withdrawn by the attending physician are examined at the laboratory and a report telephoned to the physician on request. To secure prompt and satisfactory results, the fluids should be collected in a sterile container and sent immediately by messenger to the laboratory. Fluids should never be left at the stations as delay results and the fluids are usually unfit for examination when they reach us. While we are constantly on call, it is desirable that night and Sunday calls be reserved for real emergencies as the force is small and the transportation facilities at night are far from good. By emergency cases, we mean those in which acute illness has developed within a few hours before the administration of serum. We must confess to some annoyance when we are called around midnight to see a case of tuberculous meningitis where the illness has lasted perhaps for some two weeks.

TABLE I.

NUMBER OF CASES SEEN LISTED BY YEARS 1910-1920.

	1910-1911.	1911-1912.	1912-1913.	1913-1914.	1914-1915.	1915-1916.	1916-1917.	1917-1918.	1918-1919.	1919-1920.	Total.
Epidemic cerebrospinal meningitis	18	23	30	41	38	57	85	112	78	36	535*
Tuberculous meningitis	22	45	37	55	66	75	68	94	57	52	571
Pneumococcic meningitis	3	0	5	10	8	7	6	5	6	9	59
Streptococcic meningitis	5	2	4	5	8	6	6	2	3	7	48
Influenzal meningitis	0	2	1	2	2	6	3	6	2	8	32
Staphylococcic meningitis	0	0	0	0	0	1	2	3	2	1	9
Other meningitides	1	2	0	1	1	6	7	5	2	14	39
Poliomyelitis	1	24	16	6	12	75	351	39	24	14	562
Encephalitis	0	0	0	0	0	0	0	0	39	115	154
Pneumonia	6	8	16	23	23	27	35	31	33	46	248
Other diseases	13	22	13	35	51	75	93	67	74	76	519
Normal	0	1	1	0	0	0	11	0	0	0	13
Diseases not determined	1	6	6	9	8	8	45	27	5	14	129
Total	....	....	....	....	....	....	....	....	....	....	2,918

* 17 E. C. S. M. cases counted in total number of cases but not in mortality statistics.

EPIDEMIOLOGY.

Meningitis like poliomyelitis is a disease for which the majority of individuals apparently have a natural immunity. While it is unquestionably an infectious disease, few cases give a history of direct exposure. In only 14 instances have we seen more than one case in a household. Eight of these instances were as follows:

1. The mother of a child eleven years old, developed meningitis three months after the child had recovered from the disease. It would seem impossible in this instance that the mother had contracted the disease from the child. Moreover, since the mother subsequently proved to be susceptible, it is an unexplained circumstance that she did not develop meningitis at the time when she was so closely exposed to it in caring for the child.
2. Two brothers, adults, were sick with meningitis in the same room in most unsanitary surroundings. The first patient died after an illness of five weeks. The second did not become ill until nearly two weeks after his brother, but he then developed a fulminating form of the disease and died in three days.
3. A brother and sister (adults) and the eight year old child of the sister were ill with meningitis in the same apartment. The brother and sister had become ill within two hours of one another. Both died. The child was taken ill five days later than her mother and uncle. She was treated within twenty-four hours and recovered. In another apartment of the same house a neighbor was attacked by the disease one day before the little girl. He also recovered.
4. A girl of fourteen years developed the disease in mild form and recovered. Her brother three and a half years old became sick two days later than his sister. He also had a light attack and recovered.
5. A girl nine years old had meningitis and recovered. A sister five years old was attacked four days after the elder child and died within forty-eight hours.
6. A brother and sister of two years became ill with meningitis one day apart. Both recovered.
7. Two children in a family were attacked by meningitis two months apart. Both recovered.
8. A boy of four and a half years was taken sick with meningitis and his sister seven years old developed the disease eight days later. Both recovered.

The presence of meningococci in the naso-pharynx in meningitis patients, in those associated with them, and in others who cannot be proved to have been in association with the sick has given rise to much investigation and study. This subject of carriers is discussed in an article by Dr. Williams in the present volume.*

ETIOLOGY.

The specific cause of Epidemic Meningitis as is well known is the meningococcus. In patients suffering from the disease, this organism is found in the cerebrospinal fluid and at certain stages of the disease it is found also in the blood.

The distribution of the meningococcus in various forms of extra-meningeal infection is as follows: In the lungs in cases of meningococcus pneumonia, in the pus of joints in arthritis and in orchitis, endocarditis and other purulent conditions such as otitis media, cystitis and pyelitis and in the lesions of the skin both hemorrhagic and herpetic.

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* See page 324.

As previously stated the meningococcus is found in the naso-pharynx both in healthy individuals and in those suffering from meningitis.

Among contributing factors, the season of the year is of importance. With few exceptions, epidemics of meningitis have made their appearance or have reached their climax in winter and spring. Sporadic cases show marked tendency to increase at the same seasons.

TABLE II.

INCIDENCE OF CASES OF EPIDEMIC MENINGITIS.

According to Season.

	July-October.	October-January.	January-April.	April-June.
1910-1911	..	5	3	9
1911-1912	5	4	10	5
1912-1913	4	6	9	11
1913-1914	1	4	13	22
1914-1915	3	10	12	13
1915-1916	4	6	17	30
1916-1917	10	12	18	45
1917-1918	10	10	45	47
1918-1919	19	20	18	21
1919-1920	8	10	12	6
Total	64	87	158	209

The question of age is also of importance. While meningitis occurs at all ages, a majority of the cases are found in children or adolescents. This is of course exclusive of the epidemics in army camps where the patients are necessarily all adults. In the New York City epidemics of 1905, 1906 and 1907, 65 per cent. of all cases were in children under ten years of age.

The factors of sex and race have been found to be without significance in the incidence of meningitis.

Incubation.

Since so few cases of meningitis give a history of definite exposure the incubation period is difficult to determine. Judged from the few cases of direct contagion which have been reported, the incubation period would seem to be short, perhaps three to five days as an average. Our own cases with one or two exceptions confirm this view.

Clinical Description and Classification.

The clinical picture in the early stages of meningitis may vary within wide limits. In mild cases and those of moderate severity, there is little in the early stages upon which to base a differentiation between meningitis and a number of other infections. In a case of *moderate* severity in either children or adults, there is usually a sudden onset with headache, fever and vomiting. Within twelve to twenty-four hours a stiffness of the neck develops together with a positive Kernig's sign. A petechial eruption is present in from 10 to 15 per cent. of sporadic cases. An herpetic eruption is also frequent. In these moderate cases the patient is usually irritable, rarely delirious or stuporous. The reflexes are generally somewhat increased, the increase showing equally on both sides of the body. Constipation is the rule. In mild cases with appropriate treatment, there is a tendency for all the symptoms to clear up in from one to four weeks. Such a mild beginning, however, does not necessarily lead to a favorable termination. The uncertainty of prognosis in meningitis cannot be too strongly emphasized. At any period in the course of the disease a change for the worse may take place. This may be a gradual process, the organisms persisting in the spinal fluid, the symptoms refusing to yield to treatment and becoming more accentuated. In other cases the unfavorable change may come with startling suddenness in the course of a hitherto mild illness. A basilar condition may develop with blocking off of the spinal fluid. There may be a sudden increase in temperature and pulse rate, a marked increase in the rigidity of the neck and in the degree of opisthotonos, delirium, convulsions or coma. In cases ending fatally, death may follow these changes within a few days or may be delayed for several weeks. Such a change as described, however, is not necessarily an indication for an unfavorable prognosis. In some cases it may be followed after a longer or shorter time by gradual improvement and ultimate recovery. On the other hand, in both mild and severe cases after improvement has gone on almost to the point of recovery, there is always danger of a more or less serious relapse. Such a relapse may terminate fatally, but the majority of such cases in our experience have cleared up and ended in recovery.

The above statements apply to mild or moderately severe cases of meningitis. In the *severe* cases all the above conditions are of course accentuated. In addition to the severe cases which run a more regular course, two distinct types should be considered especially—the septicaemic and the fulminating. In the *septicaemic* type, the disease presents the characteristic picture of a septicaemia rather than that of a meningitis. In the majority of these cases the meningococcus is found both in the blood and in the spinal fluid, but the symptoms take their character chiefly from the general blood infection. In certain cases, the meningococcus may fail to invade the meninges at all being found only in the blood. Strictly speaking the term meningitis is a misnomer when applied to such cases. The onset in septicaemic meningitis is usually slower than in the ordinary form of the disease and the development of meningeal symptoms may be late. A profuse hemorrhagic eruption is a practically constant symptom. Extra meningeal infections such as arthritis, orchitis, endocarditis and pneumonia are much more frequent in this type of meningitis. These cases also vary greatly in their severity, but the average mortality is higher than in the cases more strictly meningitic in character. These septicaemic cases were especially common in certain of the army camps and were admirably studied and described by Herrick[1] and others.

Except in some of the epidemics in army camps, the septic type of meningitis has been but rarely found. No organism is absolutely selective in its point of attack, but notwithstanding the relatively large number of extrameningeal infections in some of the camps it remains true that in civil life these do not often occur. When the pneumococcus invades the blood stream, it may set up an otitis media, a meningitis, or any one of half a dozen diseased conditions, but in the vast majority of cases it means pneumonia. In the same way when there is an invasion of the blood stream by the meningicoccus in the vast majority of cases (in civil life at least), it means meningitis and the complications and sequelae occurring will be by direct extension from the meninges rather than by blood-borne infection.

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[1] Herrick: Arch. Int. Med., Ap. 1919, Vol. XXXIII, pp. 409-418.
Herrick: Amer. Jour. Med. Sci., Oct., 1919.
Herrick: Clinics of North America, Sept., 1918.

The *fulminating* type of meningitis is often septicaemic in character but not necessarily so. Its chief characteristic consists in the overwhelming nature of the infection, to which the patient may succumb in from twelve to seventy-two hours from the beginning of the illness. This allows no interval for a systematic development of symptoms. The patient may simply fall as if stricken with apoplexy or there may be intense headache, vomiting and a rapidly developing stupor from which there is no rally. Rigidity of the neck and Kernig's sign may show early, but may be lost in a few hours upon the development of a condition resembling shock. Because of the occurrence of such types, the question of a possible meningitis should always be considered in cases of sudden death without apparent cause.

In the *chronic* forms of meningitis, cases may show a very prolonged course in which the final outcome may be in doubt almost to the very end of the illness. The ordinary chronic type and the relapsing type have been alluded to above. That chronic form in which a walling off of the spinal fluid develops due to a basilar meningitis, gives rise to special conditions.

Basilar meningitis is the term applied to that form of the disease in which the inflammation is more actively located at the base of the brain. In consequence of this a plastic exudate forms which closes the foramen of Magendie and prevents communication between the ventricles and the subarachnoid space. In this condition, there is an increased intracranial pressure due to the blocking off of the spinal fluid, an accentuation of the meningeal symptoms, and a pronounced opisthotonos. No fluid can be obtained by spinal puncture, repeated attempts resulting in " dry taps " and at most only a few drops of serum will run in by gravity.

Basilar meningitis may develop at any age in children or adults, but it occurs with by far the greater frequency in young children and is one of the most important causes of the high mortality in children under two years of age.

The basilar form may be due to an extension downward of the inflammation which began higher up or to an increasing intensity of the inflammatory process located originally at the base of the brain. Most cases of this condition develop in the course of a meningitis in which fluid has been obtained in the earlier punctures; occasionally a case is seen in which there is failure to draw fluid at the first puncture but such a case is usually one which has been neglected for some time and it is possible that punctures done earlier would have been more successful.

Another type of walled off meningitis is that in which the inflammatory process is so localized and cut off from the rest of the cerebro-spinal system that the organisms are not found in the spinal fluid; conversely the curative serum cannot reach the site of infection. This condition occurs usually in the course of a regular meningitis in which the clearing up of the spinal fluid is not accompanied by an amelioration of the symptoms and subsequent recovery.

Both of these forms, the basilar and the localized walling off, are generally fatal.

In children under one year of age, meningitis infections constitute a distinct class of cases which will be considered more in detail later. The symptoms at this age are often very indefinite and the diagnosis may have to depend entirely upon the examination of the spinal fluid.

It is true of all types of epidemic meningitis that the temperature runs no regular characteristic course but on the contrary shows abrupt and striking variations within a short time so that an accurate estimate is only possible when the temperature is taken at frequent intervals. In some patients the disease may run a nearly afebrile course and this may occur even in some very severe cases.

The pulse in meningitis while often slow in the early stages is generally fairly rapid when the disease is once established and in our experience is usually regular. The character of the pulse is of much more prognostic significance in meningitis than the height of the temperature.

It is of course apparent that an almost endless variety of types may be found in this disease. From our experience it has seemed to us that the cases might be generally classified as follows:

1. *Acute forms.*
    (a) Ordinary acute form (mild or severe)—in which the patient dies or is well on the way to recovery in two to three weeks.
    (b) Septicaemic (usually severe).
    (c) Fulminating type (always severe)—in which death occurs in forty-eight to seventy-two hours or less.
2. *Chronic forms.*
    (a) Ordinary chronic form lasting three weeks or over (may be either mild or severe).
    (b) The form with basilar meningitis, or other walling off (always serious).
    (c) The relapsing type (mild or severe).

The symptoms of meningitis will be considered more in detail under Differential Diagnosis.

Meningism.

Meningism is a condition which is so frequently met with and which sometimes causes such difficulties in diagnosis that it requires special description.

By meningism we mean that condition in which meningeal symptoms arise in the course of some disease and in which no invading organisms and no pathological condition can be demonstrated in the meninges.

The cerebrospinal fluid in this condition is increased in amount but clear and practically always normal in character. The few exceptions to this rule which have been found in a large number of cases have fallen mainly into four groups: First—fluids from patients with severe and prolonged convulsions notably in the convulsive type of whooping cough; second—where the underlying illness has been of long duration, especially if the patient is about to die; third—when there is an inflammation near the dura, as in otitis media, brain abscess or sinusitis; fourth—in a miscellaneous group of diseases, especially typhus fever and mumps.[2] In the first two groups the changes in spinal fluid are probably due to minute hemorrhages or to congestion and in the third group to an inflammation by extension described by Strauss[3] under the name of "Meningitis Sympathetica."

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[2] Kaunitz, Julius: Jour. Am. Med. Ass., May 18, 1918, Vol. 70, pp. 1448-1449.

[3] Strauss: Am. Jour. Med. Sci., Nov., 1917.

In a case of cerebral embolism with a septic endocarditis, there was increased albumin and globulin with normal cytology. In a case of cerebral thrombosis or embolism, there was a slight increase in cells also in albumin and globulin.

Of course tetanus and cerebral embolism or thrombosis do not represent a true meningism since in all these cases there is a definite lesion of the central nervous system; so also in a case of spastic paraplegia which showed a slight increase in albumin.

The amount of fluid obtained by spinal puncture in meningism depends upon the stage at which the puncture is done. In some cases we have withdrawn very large quantities—from 80 to 100 c.c. In other cases the fluid has been but little increased in amount. It is commonly accepted that in a true meningitis the fluid is inflammatory in character and of the nature of an exudate; on the other hand in a meningism, as we use the term, the fluid is of the nature of a transudate. According to Anglada,[4] the albumin present in normal spinal fluid is serum globulin, whereas in meningitis it is serum albumin.

The history of meningism depends of course on the underlying conditions, but the onset of meningeal symptoms is usually sudden. The condition is found complicating a great variety of diseases, but occurs with especial frequency in connection with pneumonia. In our experience of 265 cases complicated by meningism, 104 were cases of pneumonia.

In meningism there may occur as marked symptoms of meningeal irritation as in true meningitis even to loss of knee jerks, dilatation and rigidity of the pupils, strabismus or ptosis.

Some difference of opinion exists as to the use of the term meningism. It was introduced by Dupré[5] who considered the condition one of functional disturbance. Other writers have used the terms serous meningitis, pseudomeningitis, meningitis sine meningitide and meningitis infectiosa circumscripta or circumscribed meningitis. This latter term is considered preferable by Plaut, Rehm and Schottmüller.[6] Holt[7] describes a case which showed at necropsy a circumscribed meningitis. Huber[8] in an article on "Pneumococcus Meningitis and Meningism" inclines to the opinion that the pathological basis is a localized inflammation usually of infectious origin.

We have seen only two cases of generalized meningitis following meningism. The first instance was in a patient with streptococcic meningitis following extensive burns. The first fluid drawn from this patient was entirely negative. The second specimen drawn four days later, gave a culture of streptococcus hemolyticus. Our second case of generalized meningitis developing after meningism was in a patient with staphylococcus meningitis following pneumonia. In a case of meningism complicating streptococcic osteomyelitis, the necropsy revealed normal brain and meninges. A relatively large number of cases of meningism clear up promptly. In all fatal cases in our series the severity of the accompanying disease or diseases was quite sufficient to account for death. This, of course, does not prove the absence of a localized meningeal infection, but in view of all the facts we believe the condition to be, in most instances at least, a functional one probably of toxic origin.

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4. Anglada: Le Liquide Cephalo-Rachidien. Paris, 1909.
5. Dupré: Cong. Md. de Lyon, 1904.
6. Plaut, Rehm, Schottmüller: Zerebrospinalflussigkeit, Jena, 1913.
7. Holt: Am. Jour. Dis. Children, Jan., 1911.
8. Huber: Arch. Ped., May, 1914, Vol. XXXI, No. 5, pp. 328-340.

Examination of Spinal Fluid.

In all meningeal conditions the most important point in the diagnosis is the examination of the spinal fluid.

To obtain a specimen of fluid by lumbar puncture general or local anaesthesia is not needed except in rare cases such as a very delirious patient difficult to control. In such a case a general anaesthesia may be safer than the use of so much force as would be otherwise necessary. With certain nervous patients the use of either novocaine or ethyl chloride may be indicated.

We think that general anaesthesia is dangerous and local anaesthesia takes so much preparation and time that the overcoming of the pain does not compensate for the increased nervous tension on the part of the patient. Adults not acutely ill who submit to lumbar puncture for diagnostic purposes do not seem to mind much. The back is not very sensitive. If the patient is held properly—lying on the side with the knees drawn up against the abdomen, the neck bent and the back well arched so that the intervertebral space will be as great as possible, there is very little pain when the operator is skilful. Never under any circumstances do we make a lumbar puncture with the patient sitting up. Iodin is used over about four square inches immediately around the point of election for puncture and a sterilized towel through which to find the landmarks is laid over the hips.

The hands of the operator are scrubbed and disinfected with bichloride and every precaution is used to secure asepsis. An infection of the meninges by bacteria for instance the staphylococcus is followed by such fatal results that the importance of great care will be easily realized. We have never had a secondary infection of the meninges. Sometimes the skin has become infected in small children from soiled napkins. A Quincke needle, size 15 or 16, is used and the puncture is made in the mid-line through the notch most nearly coinciding with a line drawn from crest to crest of the ilium.

A piece of tubing about 15 inches long is attached to the metal connection that fits in the end of the needle when the stylet is withdrawn. To the other end of the rubber tubing is attached the barrel of a syringe. It is well to cut the rubber and insert a short piece of glass tubing near the metal connection so that the fluid flowing out or the serum flowing in may be seen. It is desirable to attach the tubing because by raising and lowering the glass container the rapidity of outflow can be regulated. A too sudden decrease in intracerebral pressure is undesirable.

Great care should be taken to have the patient securely held in a proper position. While occasionally a beginner may be perfectly successful and an experienced operator may fail at the first attempt, the average of satisfactory punctures will be about in proportion to one's experience. The most difficult type of case is that of a very young infant without increased fluid. In these cases the dura not being distended by fluid is carried in front of the needle so that as we finally penetrate it, we often hit the wall of the canal and get a bloody fluid. In some cases of this sort a very small, sharp needle seems to work better, but in general we get blood much oftener with a sharp than with a rather blunt needle. A very small needle is unsatisfactory if the fluid turns out to be purulent.

In certain exceptional cases no fluid appears after the needle has been inserted into the spinal canal and has penetrated the dura. Such an occurrence is known as a " dry tap." It may be due to one of three causes—first, an obstruction to the circulation of the spinal fluid such as the walling off described under basilar meningitis*; second, a probably lessened secretion of spinal fluid; or third, a fluid so thick and purulent that it will not flow through the needle. When probably due to the second cause the condition is usually only a temporary one and a dry tap will often be followed by one in which fluid is obtained. When the exudate is too thick to flow readily gentle suction with a syringe may be tried. If this fails a little serum will sometimes start the flow. It is well to turn the patient when making punctures so that no two successive punctures are done with the patient lying on the same side. This insures better drainage of the ventricles.

In the routine examinations of spinal fluids cytological, bacteriological and chemical studies are made. All clear or slightly cloudy fluids are centrifuged for an hour at high speed. Guinea pigs are inoculated with fluids from suspected cases of tuberculous meningitis. Smears and cultures are made from the sediment of each fluid. The sediments of clear fluids are stained by the Ziehl-Nielson method for the tubercle bacillus; the sediments of hazy or slightly cloudy fluids are stained both for the tubercle bacillus and by the Gram method, the sediment of cloudy fluids only by Gram. Special blood stains are also used in order to study the cells.

The chemical tests that we use are the nitric acid ring test for albumin and the Noguchi butyric acid test for globulin. The albumin and globulin practically always run parallel but they serve as a check on each other. The small amount of albumin and globulin present in normal fluids is noted as ± in our records. The signs +, +|, ++, +++, ++++ represent increasing amounts and serve as a rough quantitative estimation. The presence of glucose in the fluid is tested for by using an equal amount of Fehling's solution and of spinal fluid and the result of the test is marked with regard to the speed and the amount of reduction as ±, +, ++, +++. The globulin reaction and the reduction of Fehling's should not be read for at least half an hour.

The smears are examined with regard to increase in cells, character of cells, and for bacteria. The increase in cells is estimated from the smears as slight, moderate, great, very great. We do not make a cell count as routine procedure for we do not consider that the exact number of cells has more than an academic interest. We have made a fair number of cell counts in connection with the work and find that, on the whole, the results obtained by a general estimate check up fairly well with those given by cell counts.

A careful examination is made of the smears for bacteria. Occasionally degenerated organisms can be demonstrated in the smear when the culture is sterile. On the other hand the culture will sometimes be positive when no bacteria can be found in the smear.

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* See page 19.

TABLE III.

CHARACTERISTICS OF VARIOUS SPINAL FLUIDS.

Meningeal Condition.	Pressure.	Amount Usually Obtained.	Appearance.	Cytology.	Bacteriology.	Albumin.	Globulin.	Fehling's Solution.	Animal Inoculation.
Normal.	Normal.	5-10 cc.	Clear.	Very few cells.	Sterile.	±	±	+++	Negative.
Meningism.	Increased.	A. 30-40 cc. M. 100 cc.	Clear.	Very few cells.	Sterile.	±	±	+++	Negative.
Poliomyelitis.	Increased.	A. 25-50 cc. M. 100 cc.	Clear; sometimes slight fibrin web.	Rarely polynucleosis; usually lymphocytosis up to 95%. Endothelioid and polyform cells.	Sterile.	+ to ++++	+ to ++++	Rarely ++.	Negative.
Tuberculous meningitis.	Increased.	A. 30-50 cc. M. 120 cc.	Clear, fibrin web.	Lymphocytosis up to 95%; rarely polynucleosis.	Tubercle bacilli.	++ to ++++	++ to ++++	— to +++; — in 25%.	Tuberculosis in four weeks.
Epidemic cerebrospinal meningitis.	Increased.	A. 20-50 cc. M. 120 cc.	Cloudy.	Polynucleosis up to 98%.	Meningococcus.	++ to ++++	++ to ++++	+++ to —, according to severity and stage.	....
Meningitis due to other organisms.	Increased.	A. 20-50 cc. M. 100 cc.	Cloudy.	Polynucleosis up to 98%.	Infecting organism.	++ to ++++	++ to ++++	—; may be + or even +++ early.	....

A.—Average. M.—Largest amount observed.

In any purulent meningitis with a very thick exudate, the amount of fluid may be greatly decreased.

Fluids should be examined as soon as possible after withdrawal from the patient. This is true on general principles and more especially so when the meningococcus is in question as the organism autolyzes quickly and may not be recovered from the fluid unless the examination is made within twelve hours. Cultures should not be pronounced negative until after forty-eight hours incubation.

During the past few months there has been much discussion as to the possible danger of lumber puncture in certain conditions. Especially in a condition of sepsis, it has been thought by certain workers that the procedure of entering the spinal canal for the purpose of withdrawing fluid was liable to give rise to an invasion of the meninges by the organisms in the blood. This opinion has been based upon a few clinical instances in which a lumber puncture was followed by evidences of meningitis. In the majority of these cases (three out of five) there was a meningococcic sepsis existing previous to the lumber puncture. When a sepsis is present due to any infection there is always a possibility of a meningitis developing. When a meningococcus sepsis is present the development of a meningitis becomes extremely probable in any case.

Over 500 lumber punctures have been done by the meningitis division in cases of meningism. In only two of these cases has meningitis developed subsequently. In one of these patients so much time had elapsed in the interval (nearly two weeks) that, on that basis alone, it would seem that any connection between the puncture and the development of the disease would be very improbable. In view of the large number of cases of purulent meningitis which develop *not* following lumbar puncture and the very rare cases in which it has followed a puncture which showed normal fluid, it seems to us highly improbable that there is danger of meningitis being produced in this way in practice.

Another condition in which some danger has been thought to be connected with lumber puncture is that of brain tumor. Here the sudden change of pressure has been thought to be a counter-indication, but according to our experience all trouble on this account can be avoided if the head of the patient is held low during the operation.

In all cases where the fluid is not walled off, lumber puncture would seem to be far preferable to trephining as a means of relieving intracranial pressure.

In our opinion, in view of all considerations, physicians should be urged in all cases calling for lumbar puncture to resort freely to a procedure which when carefully done is attended with so little risk, and is of such vital importance in diagnosis and treatment.

Differential Diagnosis.

The clinical symptoms and the results of spinal fluid examination are both important considerations in making the diagnosis between cerebrospinal meningitis and other meningeal infections. While this is true in a general sense, however, the spinal fluid examination has by far the greater significance. In a severe and typical case of cerebrospinal meningitis or any other purulent form of meningeal infection, the clinical picture is characteristic, but many times in all these conditions cases present a very atypical group of symptoms. Even when the clinical picture of a meningitis is pronounced, there is no distinctive clinical feature by which we can differentiate between epidemic meningitis and a meningitis due to some other infection. The final diagnosis can be determined only by means of the spinal fluid examination.

Laboratory Diagnosis.

(a) Gross appearance of fluid.

A *normal* spinal fluid is perfectly clear. When drawn with the patient in a horizontal position it flows through the needle slowly, drop by drop, perhaps 10-15 drops to the minute. The rate of the flow varies with the caliber of the needle used and according to the resistance offered by the patient. Struggling and crying out will increase the flow.

In *pathological* conditions the fluid is practically always increased in amount. It may be either clear or cloudy.

A *clear* fluid increased in amount is found in tuberculous meningitis, poliomyelitis, encephalitis, syphilitic involvement of the central nervous system, brain tumor, or rarer neurological conditions and in meningism.

A clear fluid may be found very rarely in meningitis within the first twenty-four hours of the onset. A slightly cloudy fluid is sometimes found in rather acute cases of tuberculous meningitis and in poliomyelitis.

A *cloudy* fluid (with the above exceptions) usually indicates an invasion of the meninges by the meningococcus or some other pyogenic organism.

(b) Cytology.

A *normal* spinal fluid shows an average of not more than one cell to four or five fields. Very rarely the cells are somewhat increased in meningism but usually the number is the same as in normal fluid.

In *meningitis* due either to the meningococcus or to other pathogenic organisms, the cells are usually enormously increased with polymorphonuclears predominating up to 98 per cent. Very early in meningitis a fluid may occasionally be found showing so few cells as to be nearly clear although the meningococci are present. We have seen this condition in only two or three instances. In a few cases of meningitis due to other organisms, there have been but few cells with an overwhelming infection of the fluid. The smear resembles that of a pure broth culture of the invading organism.

As cases of meningococcus meningitis clear up, the number of cells in the spinal fluid decreases and the proportion of mononuclears increases. Before relying on the decrease in cells as a positive sign of improvement, it is necessary to make sure that the organisms have disappeared. Meningitis cases are almost invariably fatal when the fluid shows a rapidly decreasing number of cells with a persistence of organisms.

In *poliomyelitis* the cells are usually slightly to moderately increased, but sometimes the increase is very great. The type of cell is in most cases the mononuclear. In a few cases there is an excess of polymorphonuclears. It was once thought that the latter type of cell predominated early in the disease and that the former made their appearance later. Further study has shown the difference to be due to a type of reaction, not to a stage of the disease. Polyform and endothelioid cells are also often found in poliomyelitis.

In *tuberculous* meningitis the cells, as a rule, are moderately to greatly increased and mononuclears predominate up to 95 per cent. In a few instances in this disease with a hazy fluid, there is a preponderance of polymorphonuclears.

(c) Bacteriology.

The smears stained as noted before (p. 23) are carefully searched for organisms. Routine cultures from all fluids clear or cloudy are made on glucose ascitic veal agar neutral to phenolphthalein, this being the medium best suited to the growth of the meningococcus.

The meningococcus is not necessarily intracellular and other organisms such as the pneumococcus or the streptococcus *may* be found in the cells. Hence one should not rely on any stain except the Gram. As cases of meningococcus meningitis clear up, the organisms decrease in number and become more and more intracellular. Finally the fluid no longer gives a culture. Cultures should not be pronounced negative until after forty-eight hours incubation. Occasionally degenerated organisms can be demonstrated in the smear when the culture gives no growth. On the other hand the culture will sometimes be positive early or later in the disease when the organisms are too few to be demonstrated by smear. When the organisms are few in number, cultures and smears should be made from the *centrifuged* sediment. Examinations should be made as soon as possible after fluids are withdrawn as the meningococcus autolyzes quickly. Examinations after twelve hours are likely to be unsatisfactory.

Early cases of any form of purulent meningitis will occasionally show a perfectly sterile fluid from the first and even from the second puncture. These cases should be regarded as meningococcic meningitis and treated accordingly until the specific organism appears, since it is most desirable to give antimeningitis serum as early as possible if the case is one of epidemic meningitis.

In certain mild cases of meningitis—probably of the epidemic type—the meningococcus may never be positively demonstrated in the fluid. In purulent meningitis due to other organisms, such organisms are practically always found later in the disease if not at first.

To diagnose tuberculous meningitis in the case of a clear fluid if tubercle bacilli are not found in smears, from 5 to 6 c.c. of fluid should be injected subcutaneously into the groin of a guinea pig. One month later the pig is injected in the axilla with 1 c.c. crude tuberculin diluted 1 to 3 c.c. with normal saline. If the pig is tuberculous it is usually dead by the next morning. For proof of tuberculosis we depend upon the finding at autopsy of caseous inguinal glands, tubercles in the spleen or both. Clear fluids that have been accidentally contaminated will sometimes produce enlarged inguinal glands but tuberculin will not cause death in such animals.

Uncontaminated fluids from cases of poliomyelitis and meningism and from normal individuals are invariably negative for bacteria both by smear and culture.

(d) Chemistry.

Chemical tests of spinal fluid show that in epidemic meningitis and likewise in other purulent infections of the meninges the albumin and globulin are always increased. This increase may be from a moderate to a marked degree in different cases. In patients recovering from epidemic meningitis, the albumin and globulin usually decrease as the inflammatory reaction subsides, but the amount has seldom returned to normal by the time treatment is stopped. There may be a fairly good reduction of Fehling's in the early stage of any form of purulent meningitis, but the reaction disappears as the patient becomes worse. If a case of epidemic meningitis is rather mild, the reaction may remain good all through the disease, but it usually disappears at the height of the attack to return as the patient improves. We have come to look upon the reduction of Fehling's as being of great prognostic value.

During the summer of 1916 we made quantitative clinical studies on the various types of spinal fluids including estimation of total nitrogen, non-protein nitrogen, urea nitrogen, creatin, creatinin and sugar. Table IV gives the median and the limits of these findings.

In epidemic meningitis it was found that the nitrogenous substance decreased and the sugar increased when the patient was improving while opposite results were obtained when the patient was growing worse.

TABLE IV.

QUANTITATIVE CHEMICAL STUDIES.

		Total Nitrogen in Mgms. per 100 C.C.	Non-Protein Nitrogen in Mgms. per 100 C.C.	Urea Nitrogen Mgms. per 100 C.C.	Creatinin Mgms. per 100 C.C.	Creatin Mgms. per 100 C.C.	Sugar.
Poliomyelitis.	Median.	22.57	15.71	12.50	.400	.405	.0613
	Limits..	34.00)	24.78)	26.60)	.9609)	.495)	.1063)
		16.37)	8.91)	5.06)	.2730)	.190)	.0250)
	Cases...	25	16	17	22	11	53
Epidemic cerebrospinal meningitis.	Median.	47.25	21.93	9.25	.476	.704	Traces
	Limits..	250.00)	136.25)	53.28)	.595)	....	.0628)
		47.25)	13.00)	3.66)	.326)	....	Traces
	Cases...	30	23	26	5	1	23
Tuberculous meningitis.	Median.	25.88	13.88	7.14	.626	.649	Traces
	Limits..	34.50)	17.25)	14.18)	.7652)	.735)	.060)
		20.86)	12.82)	4.54)	.4870)	.563)	Traces
	Cases...	10	5	8	2	2	6
Normal.	Median.	15.30	13.69	8.23	.365	....	.0815
	Limits..	22.72)	22.38)	12.05)	....	....	.0923)
		9.67)	13.68)	5.35)	....	....	.0660)
	Cases...	5	3	4	1	0	6

Clinical Diagnosis.

In making the clinical differential diagnosis, the following points must be considered: temperature, pulse and respiration; headache, mental condition—irritability, delirium, stupor; convulsions; reflexes—equal or unequal, normal, increased, diminished or absent; the eyes—pupils equal or unequal, dilated or contracted, reacting to light, strabismus, nystagmus, ptosis; gastro-intestinal condition—vomiting, diarrhoea, constipation; bladder—retention or suppression of urine; hyperesthesia; paralysis or paresis; stiffness of neck; MacEwen's sign or bulging fontanelles; Brudzinski's sign; Kernig's sign; eruption—herpetic or hemorrhagic.

Certain of these signs as headache, MacEwen, rigidity of neck, Kernig, Brudzinski occur in practically all meningeal conditions and, therefore, have little value from the standpoint of differential diagnosis.

In all forms of *purulent meningitis* the onset is usually sudden and the history throws little light on the differential diagnosis. A history of pneumonia, otitis media, an operation on the nose or throat or an injury to the head may suggest a meningitis due to the pneumococcus or streptococcus rather than to the meningococcus. It is not safe, however, to be guided by such a probability, and a meningitis under these conditions should be treated as though caused by the meningococcus until proved to be due to some other organism.

The following table gives an outline of some of the chief differential clinical points between epidemic meningitis and the conditions most difficult to diagnose from it.

TABLE V.
DIFFERENTIAL DIAGNOSIS.

		Epidemic (Meningococcic) Meningitis.	Meningitis Caused by other Pus Organisms (Pneumococcus Streptococcus, etc.).	Tuberculous Meningitis.	Poliomyelitis.	Meningism.
	Previous History.	History of exposure rare.	Otitis media or sinusitis. Pneumonia. Operation on head. Injury to head.	Infection difficult to trace.	History of exposure rare.	Some underlying disease, often pneumonia.
	Temperature.	Usually fairly high and irregular.	Irregular. Often very high.	Low and irregular.	High at first. Low and irregular later.	Depends on underlying disease.
	Pulse.	Not characteristic.	Not characteristic.	Irregular in rate and rhythm.	Not characteristic.	Not characteristic.
	Respiration.	Irregular (Biot).	Irregular (Biot.)	Cheyne-Stokes towards end.	Not characteristic.	Not characteristic.
	Headache.	Marked.	Marked.	Marked.	Not persistent.	Marked.
	Irritability.	Early—may clear or go on to ↓	Early— Changes to ↓	Early—changing soon to apathy advancing to ↓	Early but usually transient.	May be present.
Mental Condition.	Delirium.	↓ or ↓	↓ or ↓	Rare. ↓	Rare.	Rare.
	Stupor.	↓	↓	↓	Rare.	Rare.
	Convulsions.	In children common, especially at onset; in adults, rare.	In children, generally present toward end; in adults rare.	Usually present at some stage.	Rare, except at onset.	May occur.
	Muscle Reflexes.	Usually increased at beginning, lost at height, return in convalescence, usually equal on both sides.	Increased at beginning, lost at height, generally equal on both sides.	May be increased early. Lost as disease progresses. Generally equal.	May be increased, decreased or lost. Often unequal on two sides.	May be present or absent. Usually equal.
Eyes.	Pupils.	Equal usually. Dilated at height. Usually fixed.	Sometimes unequal. Usually dilated. Usually fixed.	Often unequal. Usually dilated. Usually fixed.	Usually equal. Often dilated. Usually react.	Usually equal. Often dilated. Usually react.
	Strabismus.	Common.	Common.	Common but transient.	Occasional.	Rare.
	Other Symptoms.	Choroiditis.		Ptosis, transient.	Ptosis may occur.	
Gastro-Intestinal	Vomiting.	Common—early.	Common—early.	Common—early.	Common—early.	Inconstant.
	Diarrhoea.	Rare.	Rare.	Rare.	Common.	Depends on underlying disease.
	Constipation.	Common.	Common.	Common.	Rare.	Depends.

TABLE V (*Continued*).

DIFFERENTIAL DIAGNOSIS.

		Epidemic (Meningococcic) Meningitis.	Meningitis Caused by other Pus Organisms (Pneumococcus, Streptococcus, etc.).	Tuberculous Meningitis.	Poliomyelitis.	Meningism.
	Hyperesthesia.	Fairly common early.	Not so frequent.	Not so frequent.	Characteristic.	Variable.
	Paralysis.	Rare.	Rare.	Fairly common, especially facial. Transitory.	Characteristic.	Rare.
	Stiff Neck.	Marked.	Marked.	Moderate.	Variable.	Variable.
	MacEwen's Sign.*	Characteristic.	Characteristic.	Characteristic.	Characteristic.	Characteristic.
	Brudzinski's Sign.**	Common in young children.	Common in young children.	Common in young children.	Common in young children.	Common in young children.
	Kernig's Sign.†	Marked.	Marked.	Moderate.	Variable.	Variable.
Eruption.	Herpetic.	Fairly common.	Rare.	Absent.	Absent.	Absent.
	Hemorrhagic.	Fairly common.	Rare.	Absent.	Absent.	Absent.
Spinal Fluid.	Appearance.	Generally cloudy.	Generally cloudy.	Generally clear.	Generally clear.	Clear.
	Cells.	Polymorphonuclears generally greatly increased.	Polymorphonuclears generally greatly increased.	Mononuclears are generally greatly increased.	Mononuclears are generally moderately increased.	Usually not increased.
	Albumin and Globulin.	Greatly increased.	Greatly increased.	Moderately to greatly increased.	Slightly to moderately increased.	Generally not increased.
	Reduction—Fehling's.	Generally diminished or absent.	Generally diminished or absent.	Generally diminished or absent.	Generally normal.	Normal.
	Organisms.	Meningococcus.	According to infection.	Tb. bac.	Not found.	None.

* Change in percussion note over lateral ventricle, due to distension with fluid.

** Flexing of arms and legs when head is bent forcibly forward.

† Difficulty in straightening leg at knee when thigh is flexed on abdomen.

A study of this table shows that while certain clinical signs and symptoms are suggestive of certain diagnoses such a wide variety of symptoms may arise in any of the above diseases that the final diagnosis as before stated, must rest in all cases upon the examination of the spinal fluid.

Among the diseases which are especially difficult to diagnose clinically from meningitis, is a central or apical pneumonia particularly in children. The sudden onset with chills, fever and vomiting, occasionally delirium and often convulsions is very suggestive of meningitis and as shown by Table V, meningism develops in the course of pneumonia more frequently than in other diseases.

In typhus fever the abrupt onset, marked meningeal symptoms, intense headache and also the rash are quite suggestive of meningitis. The rash, however, appears later in typhus and passes through various stages before becoming hemorrhagic. The temperature is less irregular than in meningitis.

Smallpox before the appearance of the rash may also give rise to difficulty.

Fortunately both smallpox and typhus are so rare that they do not ordinarily enter into the problem of differential diagnosis. They must, however, be borne in mind.

In influenza the onset with fever, headache, pain in the back and legs, often stiffness of the neck and a slight Kernig simulates meningitis.

In typhoid fever the onset is usually more gradual than in meningitis. The rose spots may suggest the eruption of meningitis, but they are later in appearing. A blood count shows a low white count in typhoid and a moderately high leucocytosis in meningitis. Blood cultures early and the Widal test later are also of value.

Tetanus may suggest meningitis by the opisthotonos and hyperaesthesia. The fever and constitutional symptoms of the onset of meningitis are lacking in tetanus and the trismus, rigidity of the abdominal wall and characteristic convulsions of tetanus are lacking in meningitis. Very rarely trismus may occur in meningitis. No rash appears in tetanus but it may be absent also in meningitis. The mind is clear in tetanus but it may be so also in meningitis. The convulsions of tetanus are characteristic and the history of an injury is of value.

In measles, scarlet fever and whooping cough, meningeal symptoms may arise, but here the question will be to ascertain whether there is a meningitis present complicating the disease, not to decide between a primary meningitis and the disease present.

The fulminating forms of meningitis where the patient passes quickly into coma, present additional difficulties in diagnosis. Poisoning, diabetic or uraemic coma or cerebral hemorrhage may be suspected. The absence of fever in all these latter conditions will assist the diagnosis, but it must not be forgotten that the temperature in meningitis may occasionally be normal or subnormal. The history will be of value, also the examination of urine and blood. Here again we must remember that in meningitis a transitory glycosuria may occur.

In all such involved and serious conditions, lumbar puncture is so valuable an aid to diagnosis that we cannot afford to disregard it.

TREATMENT.

The treatment of epidemic meningitis resolves itself into three parts—prophylactic, specific and general.

The prophylaxis of a disease like meningitis is very difficult. As stated before, the great majority of human beings seem to be immune. On the other hand, those who are susceptible can apparently acquire the disease by a very casual association with carriers since a history of either direct or indirect exposure is to be obtained in so very few cases. The isolating of patients and of those known to be carriers has been the chief available method of prophylaxis. If, however, the susceptible individuals could be recognized and then protected by some method of active immunization as is done in the case of typhoid fever, we should have a procedure of much greater value. Some work has been done by Gay and Southard in an attempt to determine immunity to meningitis by means of a skin reaction but this is still wholly in an experimental stage. As the meningococci are found in the nose and throat and occasionally in the urine of patients, the excretions should be disinfected and those in contact with the sick should be taught how to avoid infection.

The *general* treatment of epidemic meningitis is much like that of any acute febrile disease but certain points demand special attention. The headache and restlessness must be so controlled that the patient will get adequate rest and sleep. If moderate doses of bromide, chloral or the coal tar products do not suffice to bring this about, small doses of morphia given preferably by mouth, will control the suffering and will not depress the heart. Some physicians think better results are obtained with a combination of hyoscine and morphine, thus using very small doses of each drug. The diet must be liberal as soon as the vomiting has stopped. Small quantities of liquid food should be given at frequent intervals and the amount and variety of food should be increased as rapidly as the patient can tolerate it. The necessity of keeping up the nutrition is especially urgent in meningitis as the illness may be prolonged and the chance of recovery may rest very materially on the patient's reserve strength. The vomiting, although often persistent for the first day or two, usually subsides as soon as lumbar puncture is done and does not often require any further treatment.

The bowels are often obstinately constipated during convalescence as well as at the height of the disease. During convalescence, rather large doses of rhubarb and soda (2-3 teaspoonfuls before meals) have been found to relieve this conditions to some extent.

Retention of urine occurs not infrequently at the height of the disease and distention of the bladder must be guarded against. The tendency to retention does not last usually more than two or three days.

When stimulation of the heart is indicated Maclagen and Cooke[9] have advised the use of adrenalin, 15-20 m. given every three hours by intravenous or intramuscular injection. They report cases in which this drug has been used with good results and recommend it because autopsy findings have shown hemorrhage into the adrenals in fulminating cases. We have used adrenalin in several instances. It has seemed to give effective stimulation for a time, but nearly all the cases where it has been required have happened to be those in which a fatal outcome has resulted. Caffeine sodium benzoate is also a valuable stimulant in meningitis.

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[9] Maclagan and Cooke: Brit. Med. Jour., 2, 869, Dec. 23, 1916.

The *specific* treatment of meningitis consists of the intraspinal administration of antimeningitis serum. This is a specific immune serum of therapeutic value only in meningococcic meningitis and then only when administered subdurally.

Antimeningitis serum was first used intraspinally by Jochmann[10] whose results were published in April, 1906. He recommended the removal of 20 to 25 c.c. of spinal fluid and the injection of 20 c.c. of serum, this injection to be repeated once or twice if indicated by the temperature. Flexner[11] who did much for the development of the intraspinal method, recommended that rather large doses, 30 to 45 c.c. be given, provided that as much or preferably more spinal fluid had first been withdrawn. He advised that this treatment should be repeated daily so long as meningococci could be found in smears and that in very severe cases the serum should be given every twelve hours for one or even two days.

The details of technique in making a spinal puncture will not be repeated here as they have been already discussed fully under Examination of Spinal Fluid (p. 22).

When the fluid withdrawn is cloudy, antimeningitis serum is always administered at once even though it may be considered doubtful whether the infection is due to the meningococcus. The serum will do no harm in any case and if the patient has meningococcic meningitis the earlier the serum can be given the better. Later treatment depends upon the examination of the spinal fluid.

Before introducing the serum, a sufficient amount of spinal fluid is withdrawn to reduce the pressure to practically normal. The amount of serum injected should generally not be more and preferably less than the amount of spinal fluid withdrawn. The serum to be injected should be warmed to body temperature and introduced very slowly by *gravity* under the least possible pressure. This method was introduced by Koplik[12] who pointed out that the use of a syringe is dangerous and is probably responsible for many deaths following the administration of serum.

The size of the *dose* depends very little upon the age of the patient. A very young child can usually be given 20 c.c. provided a large amount of spinal fluid has just been withdrawn. During the first two years of our work, it was customary to give larger amounts of serum for the first dose, often 30 to 40 c.c. It happened a number of times with this dosage that the patient went into shock during or immediately after the injection. Artificial respiration, hypodermic stimulation and the withdrawal of some of the fluid if the needle was still in place brought about recovery in each case. We have since given smaller doses and such occurrences have been almost unknown.

We give as a rule not more than 20 c.c. of serum for the first two or three doses, even though very large amounts of spinal fluid have been removed. If the patient does not improve the size of the dose may be cautiously increased if the serum runs in easily by gravity. In general, very satisfactory results are obtained when doses of about 20 c.c. of serum are given daily until the spinal fluid shows a negative culture after forty-eight hours incubation. If the fluid becomes sterile before the patient's condition is greatly improved, which rarely occurs, the serum should be continued but need not necessarily be given every day. Puncture for the relief of pressure may have to be done when serum administration is no longer necessary.

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[10] Jochmann: Deutsch. Med. Woch., No. 20, 1906.

[11] Flexner: Jour. Exp. Med., XVII, 1913.

[12] Koplik: Diseases of Infancy and Childhood, Phila., 1918.

Considerable differences of opinion are found among various authorities as to the amount of serum and the intervals at which it should be given in meningitis.

Netter and Debré[13] advise a daily dose of 30 c.c. of serum, as a rule, even in very young children. In severe cases, 40 to 60 c.c. of serum is recommended. They emphasize the necessity of continuing the treatment until the spinal fluid is sterile even although 25 or more doses may be necessary. They do not recommend repeating the injections oftener than every 24 hours.

Dopter[14] recommends that a quantity of not less than 20 c.c. be injected and in severe cases, large quantities. He regards 10 c.c. as insufficient even in nurslings to whom he gives 15 to 20 c.c. and sometimes even 30 c.c. He recommends a daily repetition for 3 to 4 days. If the symptoms become worse, he advises two injections a day, unless there are indications that the serum is not being absorbed. In cases associated with grave septicaemia, he suggests the intravenous injection of large doses of serum. If the case fails to improve and there are symptoms of retention of the cerebrospinal fluid in the ventricle or of the inflammation being walled off, he advises the administration of serum into the ventricle, or its injection around the base of the brain through the sphenoidal fissure.

Holt[15] recommends a dose of 10 to 15 c.c. in infants and 15 to 25 c.c. in children from 2 to 12 years old. This dose is usually repeated in 24 hours; in very severe cases in 12 hours; daily, thereafter, for 4 to 5 days. The indications for further injections are continuance of marked nervous symptoms, persistence of temperature, persistence of leucocytosis and of great numbers of polymorphonuclear leucocytes in the cerebrospinal fluid even though no organisms are found in smears and there is no growth from cultures.

Heiman and Feldstein[16] recommend injections every 12 hours in malignant cases; in cases of ordinary severity, every 24 hours for at least 3 injections; even if the case shows marked improvement, the daily injection is to be continued until the fluid is free from meningococci. In infants, they advise 10 to 15 c.c.; in adults 30 to 40 c.c.. They further recommend punctures about every five days during convalescence up to the time when the cerebrospinal fluid returns to normal character and pressure.

Koplik advises the use of rather large doses, 30 c.c., even in infants if more than 30 c.c. of fluid is withdrawn; the dose is to be repeated for three days, or until the symptoms indicate that the disease is under control. He advises against aspirating thick fluids with a syringe on account of the danger of hemorrhages.

Dunn[17] also advises the use of large doses, even 30 c.c. and if the amount of fluid withdrawn exceeds 30 c.c., he would give 30-45 c.c. of serum or even more at a dose. He injects the serum with a syringe while most other workers greatly prefer the gravity method. In cases of ordinary severity he advises a dose daily for four days, or until the spinal fluid is sterile. In severe or fulminating cases, he repeats the dose as soon as the symptoms intensify or after 12 hours if they remain the same. He advises ventricular puncture in children under 2 years if a dry tap occurs after the diagnosis of epidemic meningitis has been made. He does not advise ventricular punctures in older children, but rather the use of vaccine.

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[13] Netter and Debré: La Meningite Cerebro-Spinale, Paris, 1911.
[14] Dopter: Ann. de hyg. publ. et de méd. lég., Paris, 1918.
[15] Holt: Diseases of Infancy and Childhood. New York, 1916.
[16] Heiman and Feldstein: Meningococcus Meningitis. Phila. and London, 1913.
[17] Dünn: Pediatrics. 1917.

All these workers agree in a general way, then, on a daily intraspinal dose of serum except in very severe cases where the dose may be repeated in 12 hours. As has been noted, there is some difference of opinion as to the size of dose.

Two rather radical departures from these commonly accepted methods have been suggested of late. One method recommended is the routine administration intraspinally of 2 or even 3 doses of serum daily; the other places great emphasis on the intravenous use of serum contemporaneous, however, with intraspinal administration.

The first method, that of frequent injections of serum, has been advocated in one of the services of a large city hospital and the attending physicians on that service have been quite enthusiastic about it. In going over the records, I find that from January, 1917, up to March, 1918, ten patients were thus treated, 5 of whom died. Not all of the cases that recovered were treated by this intensive method, while some of the cases that died were evidently in a very serious condition and died within 24 hours after the serum was given. Of course, this is not a sufficient number of cases from which to draw conclusions, but the results given are not sufficiently encouraging to induce us to try the method as a routine procedure.

From a theoretical standpoint, it is difficult to say whether or not the administration of serum every 8 to 12 hours would be better than every 24 hours. Although the exact action of the serum is not known, it is believed to exert both a bacteriotropic and a bactericidal influence, the bacteriotropic action being probably the more important. In just what dilution this action may take place, how long it takes the cells to react to their maximum phagocytic power and how long this power is retained by them, we do not know. From work done by Dochez and Horn, it is probable that all the serum is not excreted from the spinal canal for about 24 hours. Of course it becomes greatly diluted before this time. It is not at all impossible, however, that cells still having a high phagocytic power may be withdrawn by a puncture made eight hours or so after the serum is administered.

We do know that the administration of serum is in a large percentage of cases followed by a reaction which begins in from two to eight hours and continues for eight hours or more as a rule. This reaction is ordinarily characterized by a rise in temperature with increased headache and restlessness. Just what relation these manifestations have to the therapeutic action of the serum is not known, but it may be questioned whether it is well to do a lumbar puncture and give more serum before this reaction has passed.

We must keep in mind the fact that we are treating the patient as well as the disease and that very frequent and large intraspinal injections may be harmful to the patient to such an extent as to overbalance any possible benefit from an increased concentration of the serum in the spinal fluid. Doing a lumbar puncture and giving serum constitute a procedure that subjects a patient to so much disturbance and discomfort that on this basis alone its too frequent repetition is not desirable.

The second of the newer methods mentioned above, namely the intravenous administration of serum, is strongly advocated by Herrick[18] who gives a very thorough discussion of this method of treatment.

Dr. Herrick bases his recommendation for intravenous administration largely upon the septicaemic nature of the disease as he encountered it at

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[18] Herrick: Arch. Int. Med., Apr., 1919, Vol. XXIII, pp. 409-418.

Camp Jackson. According to Dr. Herrick—" The meningeal aspect of meningococccus infections has usurped such a prominent place in the commonly accepted idea of the nature of epidemic cerebrospinal meningitis that important facts have been kept in the background. The very name of the disease and of the causative agent have given undue prominence to a single manifestation of a condition that, in our opinion, is very much more than a cerebrospinal meningitis. This is by no means a new idea. Others have, from time to time, expressed the opinion that meningococcus infections are primarily septic in nature and that meningitis is a secondary complication."

It is quite generally recognized and accepted that there is a sepsis in the beginning of many if not all cases of epidemic meningitis especially those cases showing a hemorrhagic eruption. But, while there are exceptions to the rule, in the great majority of cases the clinical picture speedily becomes that of a meningitis. It hardly seems fair, therefore, to consider that the " meningitis is a secondary complication " any more than it would be proper to consider the intestinal lesions of typhoid fever or the pneumonitis of pneumonia as secondary complications.

According to Dr. Herrick's statements he was relying in the first part of the epidemic on the intraspinal method of treatment supplemented in some cases by small intravenous injections. During the latter part of the epidemic, he states that he relied mainly on large doses of serum given intravenously and supplemented by an average or small amount intraspinally.

There was a lower mortality among the cases treated during the latter part of the epidemic, but two points must be considered in estimating how much this lessened mortality can be attributed to the intravenous method of serum administration. In the first place, it is to be observed that the average number of intraspinal injections given a patient during the latter part of the time was 7.2 while during the first period the average number of intraspinal injections was only 4.6. According to our experience, 4.6 injections would represent too low an average for the successful treatment of a case of meningitis unless the disease were of a rather mild type. In view of the discrepancy in the amount of dosage in Dr. Herrick's cases, the mortality in the two series does not seem fairly comparable.

In the second place, it must be remembered that the improvement in his mortality rate occurred during the latter part of the epidemic, a period at which a lessened mortality might be looked for irrespective of treatment. As was observed by Dr. Park in connection with the recent epidemic of influenza " If you wish to prove that a method of treatment is of *no* value use it during the ascending period of an epidemic. If you wish to prove that a method of treatment *is* of value use it during the descending curve of an epidemic."

The relative value of the intraspinal and the intravenous methods of serum administration can only be decided after the two methods have been used during the same period of time on absolutely parallel series of cases. Serum should be given intravenously in cases showing marked evidences of sepsis. This procedure may perhaps also be of value in aborting the disease if given early in the attack when the presence of a well marked eruption makes the diagnosis probable before other signs of meningitis develop. It is thought by us, however, that this method of administration is not indicated as a routine procedure in the more common type of cases. Certainly it should in no wise replace intraspinal administration.

In convalescent cases, the stiff neck is frequently the last symptom to disappear. If the spinal fluid has cleared up and the temperature remains normal this symptom need not be considered with regard to treatment.

In the course of meningococcus meningitis, it has happened a number of times that we have encountered a genuine " dry tap." Such an occurrence should not be thought to contraindicate the administration of serum. It is necessary, however, to proceed with the utmost caution, introducing the serum very slowly, watching that it flows in easily by gravity and being constantly on the alert for any change in pulse, respiration or subjective symptoms.

When fluid cannot be obtained by spinal puncture, the use of ventricular puncture has been highly recommended by certain authorities. We were at one time inclined to agree with these opinions but the results obtained in the few cases where we have used the method have not been such as to predispose us in its favor. As our records show, some cases giving dry taps clear up under the more conservative method of making daily spinal punctures and allowing a little serum to run in each time. It seems to us that there is a tendency to resort rather too hastily to the ventricular route in cases giving no fluid by spinal puncture. The reports of recovery after this procedure are comparatively few and it seems probable that in some of these cases there has not been a true basilar meningitis. The danger of secondary infection is probably greater in doing a ventricular than a lumbar puncture on account of the tendency of the scalp wounds to remain open and the closer proximity of the meninges to the surface. Moreover we do not know what injury may be done to the brain substance. In at least one case of recovery after ventricular puncture of which we have a reliable report, the child became a helpless imbecile. It seems, therefore, in the light of our present knowledge that ventricular puncture is not a procedure to be resorted to hastily and certainly not without very clear indications of the presence of a basic meningitis.

For a preservative in antimeningitis serum either trikresol or chloroform has been used. In the Meningitis Division we have always considered the former preservative a very satisfactory one. In consequence of the question raised by different authorities as to a possible danger in the use of trikresol, this was discontinued for a time and chloroform was substituted in its place. Serum treated in this way gave rise to so much pain that we had much difficulty in persuading patients to submit to its administration and it was finally decided to discard the chloroform and resume the use of trikresol as a preservative. At present trikresol in the proportion of two per cent. is added to all our antimeningitis serum. A discussion of the value of the two preservatives and the results of a series of comparative tests are given in an article by Neal and Abramson.[19]

The use of autogenous vaccines in epidemic meningitis has not been extensively studied. Favorable results are occasionally reported in isolated cases. It is our custom to use an autogenous vaccine in cases running a prolonged course or tending to relapse. Such treatment has not seemed to be productive of any harmful effects and in some instances it has apparently been beneficial, but we have not used it in a sufficient number of cases to make a definite statement with regard to it. It has seemed advisable to use fairly large doses beginning with a half billion and increasing to three, four or even eight billion.

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19. Neal and Abramson: Jour. Am. Med. Ass., Apr. 7, 1917, Vol. LXVIII, pp. 1035-1037.

SERUM REACTIONS.

The administration of serum by either the intraspinal, intravenous or subcutaneous route may be followed by a general systemic reaction. This is usually more or less transient leaving no permanent ill effects. In comparison with the benefit to be derived from serum treatment, this reaction as generally seen is of so slight a nature as to be entirely negligible. In only very exceptional cases are the symptoms serious enough to interfere with the further administration of serum.

The reaction following the administration of serum is not in any way confined to the use of antimeningitis serum. The same symptoms may follow in the injection of any, even normal serum. They represent the reaction of the organism consequent upon the introduction of a foreign protein.

The reaction appears in three forms at three different intervals of time: (1) an early reaction, (2) a late reaction often called serum sickness, (3) an anaphylactic reaction.

(1) The early reaction takes place usually in four to eight hours after intraspinal administration of serum. It is shown by rise of temperature, headache, a general accentuation of symptoms and occasionally by pain in the back and limbs. This reaction occurs in a fairly large percentage of cases and usually lasts from six to eight hours. If the symptoms are at all severe, they should be controlled by sedatives.

(2) Serum sickness in a varying percentage of cases follows the early reaction. It develops in from eight to twelve days after the serum injection and may appear as late as twenty-one days after. The characteristic symptom consists of an urticarial eruption with intense itching often accompanied by a rise in temperature, an increase in meningeal symptoms and occasionally by vomiting. Sometimes swelling of the joints occurs. In one case the joints of the forearm and hand and of the leg and foot become so painful that the patient was unable to move and thought he was paralyzed. Serum sickness seems to be but little influenced by the amount of serum given and judging from the time of its appearance it is apparently always caused by the first dose. One of the severest reactions we have seen followed a single dose. When the administration of serum is resumed after an interval of several days as in the case of a relapse, an accelerated reaction has been observed in a number of instances. In such a case the urticarial eruption appears in from twenty-four to forty-eight hours instead of at the end of the longer interval. This accelerated reaction also occurs with other sera under the same circumstances. The rash of either the usual or the accelerated reaction may fade and come out again every day for several days accompanied by fever.

(3) In cases showing an anaphylactic reaction, the condition develops immediately—within two to three minutes—after the administration of serum. The patient sinks into a condition of profound shock, the much studied but little understood phenomenon of anaphylaxis. Fortunately this manifestation is extremely rare and has only occurred three times in our ten years' experience.

COMPLICATIONS AND SEQUELAE.

Epidemic meningitis is not only a serious disease as regards life, but also an account of the complications and sequelae. These are greatly modified by the use of antimeningitis serum, but are still perhaps more numerous and serious than those of any other acute infection except poliomyelitis. Many of the complications run a lengthened course becoming converted into sequelae and we have therefore grouped all these conditions under one heading. In general they may be divided as follows: I. Complications not relating to the central nervous system. II. Complications involving the central nervous system.

I. Complications not relating to the central nervous system.

Respiratory.—Pneumonia is one of the most common complications seen in meningitis. It is frequently the cause of death when the meningeal symptoms are improving. It may occur either as lobar or broncho-pneumonia. Pleurisy is rare.

Cardio-vascular.—Pericarditis, endocarditis and myocarditis due to the meningococcus may occur. These complications are rare in the ordinary forms of the disease, but in the septicaemic type they are much more common. Several cases are described by Herrick.

Renal.—A transient albuminuria with occasional hematuria may occur in the course of the disease, but is not common. Cystitis may arise as the result of retention.

Joint complications.—Arthritis is not uncommon. It may appear early or late in the illness. It usually attacks only one or two joints. We have never seen a case that did not clear up readily. Rare cases going on to suppuration have been described and the injection of serum into the joint has been tried in such cases.

II. Complications involving the central nervous system. These are very numerous and may be described under seven heads:

1. Secondary infections of the meninges.
2. Motor disturbances.
3. Sensory disturbances.
4. Psychic disturbances.
5. Hydrocephalus.
6. Complications involving the special senses.
7. Miscellaneous conditions.

1. Secondary infections of the meninges.—There are frequent references in the literature to mixed infections in meningitis but the number of cases in our experience has been very small, the second infection developing generally just before death like a pre-agonal invasion. We have only once seen the meningococcus appearing in the spinal fluid simultaneously with another organism. In this case, the first five specimens of fluid from a child of nine months showed only the meningococcus; in the sixth specimen a streptococcus was also found present. In three other cases after the meningococcus disappeared from the spinal fluid, there was an invasion by another organism. In the first of these patients a child nine months of age, the spinal fluid cleared up after four injections of serum and the child appeared well for nearly two weeks. He then became suddenly ill and the spinal fluid showed a pure culture of pneumococcus. This child died of pneumococcus meningitis. In the second patient three and a half months old, the meningococcus had disappeared from the spinal fluid but the case did not clear up clinically. The child was taken to a hospital and a later puncture showed a streptococcus present in the spinal fluid. This child died also. The third patient a child of nine and a half years, was treated by us twice and was then taken to a hospital where about three weeks later a B. coli infection of the meninges developed and the child died.

2. Motor disturbances.—In our experience paralyses have been comparatively rare with the exception of paralysis of the muscles of the eye, both extrinsic and intrinsic. In one case there was a paralysis of the arm that began, became complete and cleared up within twelve hours. In several

cases there has been stiffness of the arms and legs, particularly of the legs, that has persisted for a short time after convalescence. In rare cases we have seen a monoplegia, hemiplegia or paraplegia but these have cleared up in almost every instance. Hutinel and Voisin[20] speak of hemiplegias accompanied by aphasia. They state that "the paralyses may be either flaccid or spastic and they may or may not be accompanied by muscular atrophy and sensory disturbances." It is quite possible that some of the cases of paralysis reported by the earlier writers may have been due to poliomyelitis rather than to meningitis.

3. Sensory disturbances.—Sensory disturbances as sequelae of meningitis are rare. There may be rather severe headache persisting for a time after the meningitis has cleared up. The possibility of this being due to eye strain from weakness of the muscles of the eye, should be remembered. Sciatica and a general neuritis of the lumbar region have been seen following meningitis.

4. Psychic disturbances.—It has been a common opinion that meningitis is apt to be followed by mental disability. Hutinel and Voisin report several cases of considerable mental impairment following meningitis. It has seemed to us that error on this point may have arisen from incorrect diagnosis before lumbar puncture was in general use. We have, therefore, followed up a fairly large number of patients recovered from attacks of proved epidemic meningitis and we have been led to the conclusion that mental impairment following meningitis is rare. Epilepsy has been described as a sequela but it is very rare; we have never seen a case.

5. Hydrocephalus.—This is not a very unusual development following meningitis especially in young children. According to Dandy and Blackfan,[21] this may be in consequence of either of two conditions—a blocking off of the ventricles from communication with the subarachnoid space or a diminished rate of absorption of the spinal fluid due to contraction of the subarachnoid space caused by adhesions. The first type of hydrocephalus exists of course in all cases of basilar meningitis giving dry taps and is in our experience the more common form.

6. Complications involving special senses.—Hutinel and Voisin refer to a case in which the sense of smell was lost following meningitis. We have never seen such a result. The complications affecting the eye and ear are most important. Netter and Debré give an excellent classification of the eye complications which is as follows:

First. Inflammatory and suppurative lesions of the conjunctiva, the interior of the eye and the orbit:

- a. Conjunctivitis and corneal lesions.
- b. Iritis and irido-choroiditis.
- c. Orbital cellulitis.

Second. Complications due to the involvement of the nerves:

- a. Disturbances of the internal musculature.
- b. Disturbances of the external musculature.
- c. Optic neuritis.

Of the first group of complications, conjunctivitis is common but keratitis occurs only rarely.

Irido-choroiditis is the most serious eye complication of meningitis. There is a difference of opinion as to how the infection reaches the eye; according to some it is borne by the blood while others think it proceeds by extension along the pia arachnoid of the nerve. Councilman, Mallory and

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[20] Hutinel and Voisin: Maladies des Meninges. Paris, 1912.

[21] Dandy and Blackfan: Am. Jour. Dis. of Children, 1914, VIII, 406.

Wright[22] hold the latter view and support it by autopsy findings. The process goes on to ophthalmitis, loss of sight and atrophy of the eye-ball. The loss of sight is usually limited to one eye and is generally complete. In 410 cases of meningitis, it occurred ten times. In only one instance have we seen its occurrence in both eyes. In one instance only did the inflammation subside without so much destruction and at the end of a year there was some vision present.

Orbital cellulitis is a rare development and one we have never observed.

With regard to the second group of complications, disturbances of the internal musculature are common. The pupils are usually somewhat dilated. They generally react to light early in the disease, but this reaction often becomes sluggish or is lost at the height of the attack and returns during convalescence. The pupils are occasionally unequal.

Disturbance of the extrinsic musculature is also frequently seen. Strabismus is common. It may be either external or internal. It is more often unilateral. Nystagmus occasionally develops. Complete ophthalmoplegia is said to occur. We have never seen it. Ptosis is rare. Diplopia may occur at intervals during the course of the disease.

Authorities differ with regard to the occurrence of optic neuritis in meningitis. Netter and Debré consider that optic neuritis progressing to optic atrophy with total blindness is not a very infrequent complication. It developes either rapidly rather early in the disease, or slowly in the later stages of the chronic type. In their opinion systematic examination of the eye grounds will often reveal changes in the fundus from papillary hyperaemia to papillitis and neuroretinitis. These changes may last only a short time and there may be little or no disturbance of vision accompanying them. Foster and Gaskell[23] found no evidence of optic neuritis in any of the thirty cases which they examined repeatedly. Blindness occurs sometimes with apparently normal eye grounds. Sladen[24] cities such a case. We have not examined the eye grounds in our cases. The only instances where we have observed blindness have been in the cases of iridochoroiditis previously mentioned and in basic meningitis or chronic meningitis shortly before death when the actual reality of the blindness was difficult to prove. In one other case of meningitis blindness occurred but lasted only for a short time and cleared up completely.

Of complications involving the other special senses, those of the ear are the most important. Otitis media develops occasionally in the course of epidemic meningitis. When this occurs the meningococcus is not necessarily responsible as some other organism may be found present in the ear lesion. The condition sometimes develops during convalescence.

Deafness due to inner ear involvement is the most frequent of all the serious complications of epidemic meningitis especially in children. It develops in mild as well as in severe cases. It is usually bilateral, complete and permanent. In these cases according to Councilman, Mallory and Wright, the auditory nerve shows exudative and degenerative changes due to the extension of the inflammatory process from the meninges. This process may extend until it involves the labyrinth and even the organ of Corti. The fibrinopurulent exudate soon becomes organized into a mass of osteoid connective tissue and in the final stages there is complete destruction of all the structures of the inner ear. In young children mutism follows as a natural sequence. It is difficult to be sure just when the deafness begins in very sick children but it usually develops rather early in the disease. In one of our cases it was not apparent until three weeks after the last lumbar puncture when convalescence seemed well established. Deafness then developed very suddenly and was not accompanied by any other symptom so that it could not be attributed to a relapse. In a certain small percentage of cases, the deafness is not complete and clears up almost entirely during convalescence.

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[22] Councilman, Mallory and Wright: Report of State Board of Health, Mass., 1898.

[23] Foster and Gaskell: Cerebro-spinal Fever, Cambridge, 1916.

[24] Sladen: Johns Hopkins Hospital Reports, Vol. XV.

7. Miscellaneous Conditions.—Under this head may be mentioned brain abscess which is an occasional sequela of meningitis. A hemorrhagic condition of the brain or the meninges may develop in a severe meningitis.

It must be borne in mind that different epidemics seem to be attended by different classes of sequelae and also, that the percentage of cases in which sequelae occur has been greatly diminished by serum treatment.

CASE HISTORIES.

From the records of the many cases seen during the past ten years, a few case histories have been selected as of special interest either because they present difficulties in the way of diagnosis or because they illustrate the value and the results of serum treatment.

Case I. S. K. six years old. Illness began March 10th, 1915, with pain in ear and nausea. March 11th, patient was admitted to hospital with severe headache, pain in neck and eyes, photophobia, stiffness and retraction of neck, Kernig's sign and hyperesthesia. Temperature 102.3° F., pulse 130, respiration 44. March 12th no apparent change in general condition, but a macular eruption was present and MacEwen's sign was positive. Knee-jerks were normal. Examination of the eyes was resisted.* Temperature 100° F., pulse 144, respiration 36. By lumbar puncture 25 c.c. of cloudy spinal fluid were removed and 20 c.c. of antimeningitis serum injected. The withdrawn fluid showed a great increase in cells (95 per cent. polymorphonuclear), a few Gram-negative diplococci, mostly intracellular, a +++ albumin and globulin, and an absence of glucose. A pure culture of meningococcus developed in three days.

For the next few days the condition varied slightly. Spinal fluid was withdrawn and serum injected each day. March 17th, the condition was better; 40 c.c. of much clearer fluid were withdrawn which showed one colony of meningococci in culture and for the first time there was a slight reduction of Fehling's solution. An autogenous vaccine (500,000,000) was given. For the next five days during which the serum and vaccine treatments were continued, the patient showed improvement. The spinal fluid was now sterile and otherwise exhibited considerable progress toward normal, giving a prompt reduction of Fehling and showing only a slight excess of cells, albumin and globulin. March 23rd, an urticarial rash appeared. March 24th, the temperature rose to 103.6° F. Vaccine (1,000,000,000) was given. March 25th, the temperature was still high and the patient was drowsy. Clear fluid, 50 c.c., was withdrawn, but no serum was given as the fluid continued to be sterile and nearly normal so that the failure to improve could not be attributed to the persistence of an active meningitis. In the course of the next few days during which the patient was drowsy and the high fever continued, a spastic condition developed with constant twitching on right side of face and in left arm. There was occasional difficulty in swallowing. During the first week in April there seemed slight improvement. April 12th, clear normal fluid, 40 c.c. was withdrawn. The temperature rose rapidly in the next few days reaching 108° F. on April 19th when the child died. The body showed marked emaciation and contractures. Behind the right fissure of Rolando there was a large abscess surrounded by an extensive area of hemorrhage.

Brain abscess seems fortunately to be rather an infrequent occurrence in meningitis. The few cases that have come under our observation were all characterized by marked and rapid emaciation before death.

Case II. J. G., 24 years old. Illness began suddenly September 8th, 1917, with headache, restlessness, chills and fever. When first seen on following day the patient was irritable, temperature 104° F., pulse 84, regular. There was moderate stiffness of the neck, a positive Kernig, pupils slightly contracted, equal and reacting to light, knee-jerks equal and slightly increased. Spinal fluid 35 c.c. was withdrawn which was very cloudy, showed a great increase in cells (95 per cent. polymorphonuclear) also greatly increased albumin and globulin and gave no reduction of Fehling's. The culture was sterile but the smear showed Gram-negative extracellular diplococci. Antimeningitis serum, 20 c.c. was injected. The second specimen of spinal fluid obtained on the following day, showed a pure culture of meningococcus. Lumbar punctures were made daily, 20 to 50 c.c. of fluid being removed and 20 to 35 c.c. serum injected. There was gradual improvement, the temperature became normal, the fluid became clearer and again reduced Fehling's, but the cultures remained positive until the 15th when there was no growth. September 19th, the fever and headache returned. Spinal fluid 45 c.c., slightly cloudy, was withdrawn and 35 c.c. serum injected. The culture showed three or four colonies, but no more punctures were done for five days. This was in accordance with the theory of some physicians that after a fairly large amount of serum has been given the system can take care of a few remaining organisms. This does not agree with our views but it seemed best to test the theory in this case. The patient's temperature remained normal until the 24th when he became worse and the temperature rose to 103° F.

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* An hypopyon of the left eye was observed a day or two later.

Punctures were now done daily, 40 to 45 c.c. fluid being removed and 20 to 35 c.c. of serum being given. With one or two exceptions these fluids all gave cultures of meningococcus and the reduction of Fehling's had gradually disappeared. September 28th, autogenous vaccine (500,000,000) was given. The injection was repeated every two or three days and the dose was increased to 4,000,000,000. October 6th and 7th, the fluids drawn were sterile and showed fairly good Fehling's reductions. The patient's condition was remarkably good considering the length of the illness. He was occasionally delirious at night, but seldom had fever except during the serum reaction. October 9th, 30 c.c. of nearly clear fluid were removed and 20 c.c. of serum were given. This fluid gave one colony of meningococcus in culture. Vaccine (6,000,000,000) was given. On the next three days no puncture was done. On October 13th, following a rise of temperature, 35 c.c. turbid fluid were withdrawn and 30 c.c. serum administered. Vaccine (8,000,000,000) was given. The fluid showed some growth. The vaccine caused considerable reaction. Another dose of vaccine (10,000,000,000) on the 15th was also followed by some reaction. Fluids drawn on the 14th, 16th, 17th and 18th were sterile and showed good reduction of Fehling's, the last two practically normal. Although the patient was considerably emaciated he continued to improve after this and made a rapid and complete recovery.

This case illustrates very well the necessity of continuing the serum treatment as long as the condition of the patient demands it. In all, twenty-eight doses of serum were given and recovery was apparently due to persistence in administering it and perhaps to the additional action of the vaccine. Whenever the treatment was stopped, the condition promply got worse until treatment was resumed.

Case III. M. D., three years old. Onset October 30th, with vomiting, irritability and fever. A purpuric rash appeared on the body and the joints of the left wrist and thumb became swollen and tender. On November 3rd, a blood culture showed meningococci. There was a septic temperature ranging from 98° to 105° F. The meningeal symptoms were only slight, but a lumbar puncture was done November 5th and the 30 c.c. of bloody fluid withdrawn were thought to be hemorrhagic by the physician doing the puncture. About 30 c.c. of serum were given by gravity. The child immediately became cyanotic and pulseless, but revived under the usual stimulants. There was no growth from the spinal fluid. The blood in the fluid interfered with other tests. The temperature continued high though less septic than at first but the child's evident intolerance for serum made it inadvisable to try intravenous administration. The rash was fading by November 7th though the joints continued swollen and painful. On the 9th, there was swelling of the face and the urine showed an acute nephritis. On the 14th a blotchy rash evidently a serum rash, appeared on the body and lasted for several days. The temperature was lower from the 11th to the 14th but on the 15th it was again 105° F. The temperature sank again from the 17th to the 23rd when it rose to 104.2° F. Although there were no well-marked signs of meningitis, a second lumbar puncture was done on the 24th, and 30 c.c. of nearly clear fluid were withdrawn but no serum was given. The fluid showed great increase in cells (85 per cent. polymorphonuclear), ++ albumin and globulin, +++ Fehling's and no meningococci by smear or culture. This might well represent fluid from a recovering case of meningitis. The septic temperature continued and on December 1st, a third lumbar puncture was done and 30 c.c. of distinctly cloudy fluid were withdrawn. Serum 20 c.c. was again given notwithstanding the risk of reaction as desperate measures seemed to be warranted. A severe reaction again followed and no further attempts were made to give serum, although this latest fluid showed meningococci both by smear and culture. On the following day, however, the temperature dropped to normal. The use of autogenous vaccine was begun two days later with a beginning dose of 250,000,000. This vaccine was given every other day for five injections running the dose up to 1,500,000,000. The temperature continued practically normal and recovery took place. On December 11th, urine examination showed that the nephritis had cleared up.

In this case the symptoms of sepsis were much more prominent than those of meningitis. The value of the vaccine could not be accurately estimated as the temperature had dropped following the intraspinal injection of serum and was still normal two days later when the vaccine treatment was begun. The vaccine treatment may have prevented a recurrence of symptoms and it would probably have been well to have used it earlier. The case was complicated by the acute nephritis. Whether this was due to the disease or to the serum treatment, it is impossible to say. Another striking feature of the case is the marked anaphylactic serum reaction. This is one of the very few cases in our experience where the hypersensitiveness has been so great that the continued use of serum was impossible.

Case IV. M. M., twenty-one months old. Seen first June 5th, 1916. There was a history of illness extending over two months beginning with an abscess from broken-down glands on the left side of the neck followed by a discharge from the right ear. Later, there were symptoms of some pulmonary condition evidently not a frank pneumonia and still later iritis and hypopyon of the right eye developed together with rather indefinite meningeal symptoms. The child was sent to a hospital for diseases of the eye and some general improvement followed. A lumbar puncture was not done. The eye condition was not greatly improved. She had been home from the hospital only a short time when she became worse and vomited very persistently. When we saw her she was stuporous. No nourishment had been retained and she had refused food for twenty-four to thirty-six hours. There was slight stiffness of the neck and a marked MacEwen. The knee-jerks were not obtained. The left pupil was dilated and did not react to light. The right was contracted and the tension of the eyeball markedly diminished. The temperature was 99° F. and the pulse 140, regular. The temperature had been running a rather low, irregular course. The child was greatly emaciated.

The diagnosis of this case presented several difficulties. Clinically, it looked like a tuberculous meningitis because of the low irregular temperature, stuporous condition, the late vomiting (usually an early symptom in epidemic meningitis), the emaciation and the slight but well-marked stiffness of the neck without retraction of the head. Further, there was the history of broken-down cervical glands and the indefinite infection of the lungs, all suggesting a tuberculous condition. On the other hand, there was the history of hypopyon which we had never seen in tuberculous meningitis, but which occurs occasionally in the epidemic form and the fact that the pulse though rapid, was regular while in tuberculous meningitis the pulse is usually irregular both in rate and in force. Again, the history of a running ear would suggest the possibility of a meningitis of otitic origin probably caused by a pneumococcus or a streptococcus.

We did a lumbar puncture which yielded 35 c.c. of slightly cloudy fluid and 20 c.c. of antimeningitis serum was immediately injected. The fluid withdrawn showed a great increase in cells (95 per cent. polymorphonuclear), a few Gram-negative cocci both intra- and extracellular, albumin and globulin ++++ and Fehling's +. Meningococci grew in pure cultures. Daily punctures were made with injections of serum from June 6th to June 11th. At the end of this time the fluid was sterile and the reduction of Fehling's normal. The general condition had improved.

On June 30th, as the child had been restless and had not slept well for two or three nights, a puncture was done to relieve pressure; 35 c.c. clear fluid were removed and no serum given. This fluid still showed considerable increase in albumin and globulin, though the number of cells (95 per cent, mononuclear) was only slightly increased, and the reduction of Fehling's was practically normal. Although the meningitis had cleared up and the child took nourishment well, the nutrition did not improve and she remained greatly emaciated.

Early in July on account of the continued emaciation, the patient was sent to a hospital for babies to see if the nutrition could not be improved by suitable feeding. She did improve somewhat during her stay in the hospital, but was still greatly undernourished when seen at her home in August. An excessive growth of hair had developed on the extensor surfaces of the arms and legs and on the back. Soon after this she began to improve, gained in weight and strength and began to walk and talk again. As the nutrition improved the excessive growth of hair began to disappear.

When seen in January, 1917, she was a very healthy well-developed child, intelligent and playful and seemed quite normal in every way except for the loss of sight in her right eye. The right eyeball was atrophied. The superfluous hair had disappeared.

We have seen this excessive growth of hair associated with profound malnutrition in one other case that came under our observation late and gave a history strongly suggesting an attack of meningitis. Netter and Debré are apparently the only writers mentioning this condition. They make no suggestion as to the cause. It may very well be due to some disturbance of the internal secretion resulting from the long-continued meningitis.

Meningitis in Children Under One Year.

Children under a year old constitute the most difficult cases of epidemic meningitis with which we have to deal. In the first place, the diagnosis is very difficult. Not only are we unable to elicit the patient's own sensations, but the objective symptoms are usually atypical and the most characteristic symptoms, stiffness of the neck and Kernig's sign, are so slight as to leave one doubtful of their presence. Moreover, the child is irritable and resists all handling so that it is practically impossible to tell whether one is encountering an active resistance or a resistance due to a real hypertonicity of the muscles. The Brudzinski sign is of some value, more perhaps than the Kernig, but it is not so reliable as it is in older children. It will be remembered that two signs are described by Brudzinski, the neck and leg signs. The neck sign is more commonly tested. The child should be placed on the back with the legs extended. When the head is forced forward, the legs are flexed. The leg sign may show itself in either of two ways. When one leg is passively flexed the other may be flexed also—the identical contralateral reflex—or it may be extended—reciprocal contralateral reflex. The stiffness of the neck is best elicited by either holding the child on its side or laying it on a flat table. In this way a tendency for the head to fall back is most readily brought out and observed. The reflexes are very unreliable. Convulsions are present in a rather large percentage of cases, but convulsions are present in so many conditions in children of this age as to be of little diagnostic value. A rash either hemorrhagic or herpetic is unusual in young babies. The onset is gradual in a large percentage of cases so far as can be learned from the history. Heiman and Feldstein state that in the majority of their cases the onset is sudden, but Netter and Debré comment on the insidiousness of the onset being the rule in contradistinction to the abrupt onset in adults and older children.

Netter and Debré mention irregularity of the pulse and respiration as being of value. It has not been our experience that cases of epidemic meningitis have as a rule an irregular pulse. Of course, sinus arrhythmia is well marked in children of this age. According to Holt regular rhythmic respirations are not established until the end of the second year. Therefore we have not been able to attach much importance to irregular respiration in infants.

There is usually gastro-intestinal disturbance of some sort—greenish, slimy stools more frequently perhaps than vomiting, though vomiting is often present. This condition serves to cloud the diagnosis as it may seem that all the symptoms are referable to it.

The best diagnostic points are the bulging fontanelle, the hyperesthesia and the tendency of the head to fall back. Fever is usually present also. A distention of the veins of the head is often associated with increased intracranial pressure. While increased pressure is almost always associated with a bulging fontanelle, we have seen a few cases in which a large amount of spinal fluid was obtained although the fontanelle did not bulge. According to Hutinel and Voisin, tetany may be the only clinical manifestation.

In view of the difficulties of diagnosis, one can do no better than follow the advice of Debré—"In times of epidemic think of meningitis and do a puncture on every infant sick without apparent cause." We might add that even when an epidemic is not prevailing it is safer when there is a question as to the advisability of a lumbar puncture to do the lumbar puncture.

The serum treatment for infants is in no way different from that for older children. A daily lumbar puncture should be done and the serum administered until the fluid becomes sterile. After this, lumbar puncture may be done and serum given if the clinical symptoms indicate it.

Some writers have a table of dosage arranged in accordance with the age. In our opinion the dose of serum depends on the quantity of fluid obtained and the ease with which the serum runs in by gravity, rather than on the age. Serum dosage does not need to be regulated by the body weight as does a toxic drug. The chief point of caution is to avoid increasing unduly the intracranial pressure. Usually we inject 20 c.c. of serum if as much or more fluid has been obtained and if the serum runs in easily by gravity. Netter and Debré, Dopter, Dunn and Koplik advise the use of 30 c.c. or more. Holt and Heiman and Feldstein recommend smaller doses, 10 to 15 c.c.

In performing lumbar puncture and administering serum, it is especially necessary in the case of infants to observe very carefully the pulse and respiration. It is in children under a year of age that most of the fatal accidents following lumbar puncture are reported. We have had two such experiences. One of these was in a child two months old that had been severely burned by being placed in a mustard bath. The child died immediately following the third lumbar puncture; 35 c.c. of fluid had been withdrawn and 20 c.c. of serum injected. This had been the dose of serum on the two previous occasions. The child had seemed to stand the puncture well and the burns were being dressed by a member of the family when it was noticed that the child was becoming cyanotic. She was found to be pulseless and the attempts at stimulation, artificial respiration, etc., were without results. The second case was in a child four months old. An injection of serum had been given two days before the child was brought to the hospital. During the afternoon it had seemed worse, had not nursed well and had vomited. The pulse and respiration had been increasing. Thirty-five c.c. of fluid were removed and about 15 c.c. of serum injected. The heart suddenly stopped beating and did not respond to energetic and immediate stimulation. Since in both instances the quantity of serum injected was much less than the quantity of fluid removed it does not seem probable that increased intracranial pressure could have been a factor in the results. As both these children had received serum shortly before, death could not have resulted from anaphylactic sensitiveness to the serum. In considering instances of this kind two points must be kept in mind, first, that sudden death in young infants apparently well is not, according to Holt, a very uncommon occurrence, while in children with an enlarged thymus or of the status-lymphaticus type a very slight cause is often followed by fatal results. Symmers[25] has pointed out that meningitis is often prone to be rapidly fatal in cases of status lymphaticus. Again, Ker[26] comments on the suddenness with which death took place in epidemic meningitis before serum was used. He says, " The end is often sudden, the condition of the patient changing for the worse with little or no warning, the pulse, for instance, remaining reasonably good and the respiration easy until five minutes to an hour before death when the patient collapses and in a few seconds is pulseless. He may rally a little if appropriate treatment is employed, but the improvement is only slight and does not, as a rule, last long."

In a case of status lymphaticus or enlarged thymus, it is possible that the relatively slight shock attending lumbar puncture and the administration of serum might be sufficient to hasten such a fatal collapse as described by Ker. However, in view of the large number of lumbar punctures performed with safety, it does not seem reasonable to hesitate because of the remote chance of encountering such a case. We might with as much reason refuse to remove tonsils or adenoids or perform any other simple operation although in any of these cases there may be a fatal outcome in an unusual type of child. Especially does this reasoning apply when one considers the grave risk of omitting or delaying a lumbar puncture in a child suffering from meningitis.

Not only is the diagnosis difficult in the case of young infants but the prognosis is very grave. Before the use of serum the mortality ranged, according to Flexner, from 90 to 100 per cent. Holt states that of twenty-four cases at the Babies' Hospital twenty-three died. This high mortality has been greatly reduced by serum therapy, but it has by no means reached the gratifying figures obtained in older children. Heiman and Feldstein give a mortality of 64.2 per cent. in fourteen cases. Netter and Debré have a total mortality of 61 per cent. in eighteen cases. Correcting this by omitting cases moribund when seen or suffering from some serious complication, they obtain a mortality of 46 per cent. Dopter gives a mortality of 48.6 per cent. The number of cases is not stated. Flexner reports on the case histories of one hundred and twenty-five infants among whom the mortality was 49.6 per cent.

Some of the reasons for the high mortality in infants may be considered. One cause is the frequency with which pneumonia occurs in connection with the meningitis. This has happened in six of our fatal cases. Another and more important factor is the tendency of children of this age to develop a basilar meningitis. In eleven of our fatal cases this has happened.

The following cases have some points of interest:

Case I. A. S., five months. First seen May 27th, 1918. During the preceding month the child had had bronchopneumonia which had cleared up, but had been followed by whooping cough which was still active. For the past week or ten days her temperature had been normal. She had been vomiting for five or six days, but this occurs so commonly with whooping cough that it did not seem especially significant. When examined it was observed that the fontanelle was bulging and the veins of the head were distended. The knee jerks were present and equal. The pupils reacted to light. The temperature was normal, pulse rapid (about 140) and regular. There was no rash. Altogether except for the bulging fontanelle, there was little to suggest meningitis. However, on lumbar puncture 35 c.c. of very cloudy fluid were withdrawn.

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[25] Symmers: Amer. Jour. Med. Sci., July, 1918.

[26] Ker: Infectious Diseases. London, 1919.

This fluid showed a very great increase in cells (95 per cent. polymorphonuclear), Gram negative cocci in the smear (both extra- and intra-cellular), albumin and globulin four plus, reduction of Fehling's negative. The culture did not grow. Antimeningitis serum 20 c.c. was injected.

May 28th. Condition better. Vomiting nearly stopped. Thirty-five c.c. cloudy yellowish fluid were withdrawn and 20 c.c. of serum injected. On examination this fluid was similar to the preceding specimen except that meningococci were present in the culture as well as in the smear.

May 29th. Condition good. Twenty c.c. of cloudy, yellowish fluid were withdrawn. This showed organisms in the smear, but a negative culture, and gave a faint reduction of Fehling's. Serum, 20 c.c. injected.

May 30th. The fontanelle was not bulging and the condition was much improved. A small amount of yellowish fluid was obtained by lumbar puncture and four or five c.c. of serum were injected. The fluid was negative by smear and culture, showed a slight increase in the reduction of Fehling's and a decrease in the percentage of polymorphonuclears.

The child's condition continued to be excellent and she made an uneventful recovery.

It is interesting that throughout the course of the meningitis, the temperature was normal.

Case II. S. F., ten weeks old. Seen first August 23rd, 1918. There was a history of illness since August 14th—fever, diarrhoea and restlessness. There had been no vomiting. On August 21st there had been a convulsion. On examination the fontanelle was found to be bulging. The tendency for the head to fall back was very slight. The pupils were unequal, but reacted to light. The knee jerks were not obtained. The temperature was 102° (there had been an elevation of temperature constantly since August 14th—104°-106°); pulse around 180, regular. There were marked hyperaesthesia and irritability. On lumbar puncture 18 c.c. of cloudy fluid were withdrawn. This showed a great increase in cells (90 per cent. polymorphonuclear), albumin and globulin four plus, a faint trace of reduction of Fehling's solution and the presence of meningococci both by smear and culture.

August 24th. Temperature normal for the first time since illness began; condition otherwise about the same. Had been very restless during the night. Thirty c.c. of cloudy fluid were withdrawn and 20 c.c. of serum injected. This fluid was similar on examination to that of the 23rd. Only one colony of meningococci grew.

August 25th. Condition slightly better. Twenty-five c.c. cloudy yellowish fluid were withdrawn and 20 c.c. of serum injected. The withdrawn fluid was negative by smear and culture and there was a positive reduction of Fehling's.

August 26th. Condition much better. Fifteen c.c. of yellowish fluid were withdrawn. This fluid was also negative by smear and culture. The percentage of polymorphonuclears had fallen to 80 per cent. and there was a ++ reduction of Fehling's. Serum injected, 15 c.c.

This is one of our youngest cases, and the youngest case that we have treated successfully. The prompt response to serum, the immediate fall in the temperature and the rapid improvement were most gratifying.

It will be noted that in these two cases the reduction of Fehling's while absent at first, gradually returned reaching "plus" in A. S. and "double plus" in S. F. We have observed that as a general rule the return of the reduction of Fehling's (indicating of course a return of the glucose content), is accompanied by a good prognosis. This rule, of course, is not absolute.

Prognosis and Mortality.

Meningitis has been greatly modified by the serum treatment not only concerning the final outcome, but also with regard to the whole course and duration of the disease.

The response to serum treatment may be very prompt in the ordinary acute form of the disease. After a single dose, there may be " all the difference between night and day " to quote the husband of a patient. This patient when first seen twenty-four hours after the illness began was mildly delirious and crying out with headache. She had a temperature of 103° F.

and was vomiting repeatedly. The next morning after an injection of serum she was rational and fairly comfortable. Her recovery was rapid and uneventful. In most cases the progress is slower, the patient showing a gradual improvement and five to ten days elapsing before he is comfortable and has a normal temperature. Perhaps about 20 per cent. of the fatal cases die between the third or fourth and the tenth day of the disease. From the descriptions written before serum was used, one gets the impression that cases lasted three months quite frequently and that they sometimes ran four to six months. A few of our fatal cases, generally those treated late, have lived for about three months, but the duration of the usual chronic cases under serum treatment is usually one to two months. Ker reports a case recovering at the end of 105 days and Holt reports one recovering at the end of 84 days and another at the end of 102 days. According to Holt, the usual duration of cases before the use of serum was from three to six weeks; whereas in 220 cases receiving serum, the average duration of active symptoms after the first serum injection was 11 days. Probably on an average this would be 15 to 16 days after the beginning of the illness.

Ker states that without serum treatment the percentage of relapses is as high as 15 to 20 per cent. The percentage of relapses in cases under serum treatment is only about one to two per cent.

The mortality figures of epidemic meningitis have been just about reversed since the introduction of antimeningitis serum. While previous to this time 70 to 80 per cent. of patients died and 20 to 30 per cent recovered, we now have 20 to 25 per cent. dying and 75 to 80 per cent. recovering. The exact mortality before the advent of serum treatment is sometimes questioned. In certain comparatively recent epidemics the mortality has been fairly well established as in the New York epidemic of 1905 where there was a mortality of 76 per cent. in 1,780 cases. The mortality in Belfast was over 70 per cent. before serum was used. This was reduced to 30 per cent. by Robb through the use of serum. Cases occurring at the same time not treated with serum still showed a mortality of over 70 per cent., a sufficient evidence that the general severity of the epidemic had not decreased. In Edinburgh, according to Ker, the death rate before the use of serum was over 80 per cent.

Four factors may be considered as having an important bearing on the prognosis. First, whether cases are sporadic or epidemic, and if of the latter class, the period of the epidemic at which they occur. Second, the age of the patient. Third, the presence of certain unfavorable symptoms. Fourth, the period of the disease at which serum is administered. In general, sporadic cases are considered less severe than cases occurring during an epidemic, especially during the early part of an epidemic. It is a generally accepted fact that cases are less severe during the latter part of an epidemic than at its beginning. The age of the patient has a very definite bearing on the prognosis. With children under a year old we have not been able to maintain a better mortality than about 45 to 50 per cent. However, this is a great improvement upon the mortality rate before the use of serum when it was practically 100 per cent. in children at this age. Certain symptoms are generally regarded as indicating a bad prognosis. Netter and Debré give the following: loss of consciousness, violent delirium, complete insomnia or profound stupor, an adynamic state, convulsions (especially in older children and adults), carphology, generalized trembling, purpuric eruption. Much depends on the length of time these symptoms persist. The prognosis is not unfavorably affected by high temperature, marked rigidity of the neck or severe headache. Netter and Debré consider irregularity of the pulse as of no consequence. Personally, we do not like to find an irregular pulse unless it is evidently due to a sinus arrythmia, though we do not regard it with the same concern as a rapid pulse of poor quality.

One of the most important factors in the prognosis is the date at which treatment is instituted. The following table is taken from Heiman and Feldstein.

SERUM TREATMENT BEGUN.	LEVY.			NETTER.			FLEXNER.	DOPTER.
	No. of Cases.	Deaths.	Percentage of Mortality.	No. of Cases	Deaths.	Percentage of Mortality.	Percentage of Mortality.	Percentage of Mortality.
1st day..........	6	...	.....	..	..	.....	.....	.....
2nd day..........	33	3	9.09	44	9	20.9	18.1	8.20
3rd day..........	37	7	18.91	..	..	....	....	.....
4th day..........	24	4	16.66	..	..	....	....	.....
5th day..........	14	3	21.43	32	11	33.3	27.2	14.40
6th day..........	11	3	27.27	..	..	....	....	.....
7th day..........	9	3	33.33	..	..	....	....	.....
After 1st week.....	26	7	26.92	23	6	26	36.5	24.1

Our mortality statistics from July, 1910, to July, 1920, are given below classified according to years, according to age and according to time between onset and beginning of treatment.

TABLE VI.

MORTALITY IN EPIDEMIC MENINGITIS DURING PAST TEN YEARS, 1910-1920.*

	Total No. Cases	Patients Recovered	Patients Died	Result Unknown	Mortality Per Cent
1910-1911................	17	10	7	0	41
1911-1912................	24	7	14	3	66
1912-1913................	30	17	12	1	41
1913-1914................	41	28	13	0	31.7
1914-1915................	38	25	13	0	34
1915-1916................	57	40	17	0	29.8
1916-1917................	85	65	20	0	23.5
1917-1918................	112	90	22	0	19
1918-1919................	78	62	16	0	20.5
1919-1920................	36	18	18	0	50
	518	362	152	4	29.5

* July to July.

It will be seen that the average mortality runs very irregularly when estimated in connection with a small number of cases. In contrast with the 50 per cent. mortality in 36 cases from July 1, 1919, to July 1, 1920, we have had since July 1, 1920, only one fatality among 19 cases. A total of at least 75 cases is needed as a basis for a fairly accurate estimate.

In several instances the fatal outcome was due to some intercurrent complication such as a heart lesion or pneumonia. In other instances the cases were moribund when first seen and occasionally we were hampered by a failure of cooperation on the part of the family or the attending physician.

Our average mortality is also heightened by the relatively large number of children under two (46) and especially under one year of age (102) in whom the mortality rate is always high as shown by the Table VII. Of the total number of cases, 518, seen during the past ten years, 148 or 26.4 per cent., were under two years of age.

TABLE VII.

MORTALITY IN EPIDEMIC MENINGITIS.

According to Age.

Age.	Cases.	Mortality.
Under 1 year	102	46%
1 to 2 years	46	31%
2 to 5 years	89	16.8%
5 to 10 years	108	25.9%
10 to 20 years	84	23.8%
Over 42 years	80	31%
Unknown age	9	33%

In dealing with sporadic cases, the mortality among patients seen during the first three days is likely to be relatively high. This is because these are cases of severe type; otherwise meningitis would not be suspected and we should not be called upon so early.

TABLE VIII.

MORTALITY IN EPIDEMIC MENINGITIS.

According to Time of Beginning Treatment.

Time After Onset.	Cases.	Mortality.
1 to 3 days	200	23.5%
4 to 7 days	137	27.7%
7 to 14 days	63	37%
2 to 3 weeks	47	47.8%
Over 3 weeks	22	40.8%
Onset unknown	49	27.6%

INFANTILE SCURVY.

(IV.) The Therapeutic Value of Yeast and of Wheat Embryo.*

Alfred F. Hess.

Although we possess in orange juice a specific for infantile scurvey, it is well worth while to look about for other therapeutic agents which are potent in this disturbance. This is desirable from several points of view. In the first place, orange juice cannot be considered an ideal antiscorbutic food, as it is expensive and sometimes difficult to procure, but more especially because it cannot well be embodied in the food. As a result of its having to be prepared and given separately, it is frequently forgotten by the physician or by the nurse, with the result that we encounter cases of latent or subacute scurvy which have come about through this oversight. Furthermore as long as the true nature of the antiscorbutic vitamin is unknown, we shall gain valuable knowledge of the essential chemical substance which forms the basis of this disorder by ascertaining what substances do or do not possess antiscorbutic power. It was with these objects in view that yeast was resorted to in the prevention and cure of certain cases which have been reported in other connections. As is well known, yeast has been found to act as a specific in the prophylaxis and cure of beriberi, a disease which develops generally as the result of a diet of polished rice. In a previous paper[1] dealing with scurvy the intimate clinical relationship between these two deficiency diseases was pointed out. It was shown that in both there is involvement of the heart as well as of the central nervous system. Further observations have served only to strengthen our conviction of the group relationship between these two nutritional disorders. We hope to discuss in greater detail this clinical similarity at another time; however, it is evident that we might well expect that yeast would also prove of value in infantile scurvy.

The diets of the infants on whom we wish to report consisted of preparations of pasteurized milk, with the addition of cereal in the case of those over 6 months of age. The milk was of excellent quality and had been pasteurized at 145 F. for thirty minutes. The only change which was made in the diet was the omission of orange juice, and the substitution of yeast in its stead, or in the case of very young infants who had not yet received orange juice, yeast was introduced into the dietary. The yeast was a fluid preparation, which was autolyzed and prepared as follows: As soon as it was obtained from the brewery it was pressed to a consistency of putty, and was then allowed to autolyze in a thermostat for forty-eight hours. It thus became a fluid of syrup consistency, and as it contained considerable solid matter, composed mainly of the walls of the erupted yeast cells, it was diluted somewhat and filtered. It had a final specific gravity of about 1.04. One or two tablespoonfuls of this fluid was added directly to the day's feeding of milk, or, in case the milk was not well taken, was given with a spoon.

The instances in which the yeast was given instead of orange juice to infants 6 months of age or under, in order to prevent the occurrence of scorbutic symptoms, are naturally the most interesting and significant, for it is evident that if it failed as a prophylactic, it could hardly prove to possess curative powers. We may summarize our experiences with regard to the efficacy of this therapeutic agents by the statement that our hopes proved to be unfounded, as the young infants developed some signs of subacute or latent scurvy in spite of receiving the autolyzed yeast. The symptoms were not marked, and were promptly counteracted by the administration of orange juice; however, they were sufficiently definite to enable one to make a reliable diagnosis. The weight charts of two of these cases are appended (Charts 1 and 2), but must be regarded as unsatisfactory proof of the condition, since failure to gain in weight is but one sign of incipient scurvy, in some cases by no means the most significant one; in others, as pointed out elsewhere, this sign was entirely lacking. It will therefore be well to add a few details regarding these cases. The case portrayed in Chart 1 was that of a fairly well-nourished infant who had gained steadily until it was 5 months of age, when it weighed about 11¼ pounds. It had been on Schloss milk[2] and had gained 3 pounds in the preceding two months. As it had hardly gained anything in the three weeks just elapsed, yeast was given in the beginning of April, as the chart indicated. In spite of the fact that yeast was continued for seven weeks, the baby's appetite became very poor. The yeast was then given by spoon. The child failed to gain in weight and in May manifested tenderness of the lower end of the femur. All these signs and symptoms yielded promptly when orange juice was substituted for yeast.

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* Published in Am. Jour. Dis. Children, 1917, 13, p. 98.

[1] Hess, A. F.: Jour. Am. Med. Assn., 1915, 65, 1003.

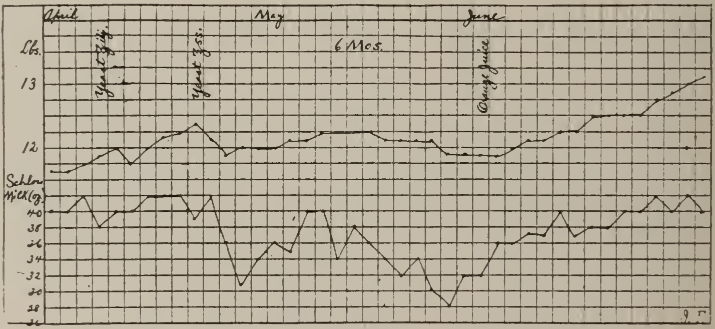

Chart 1.—Failure of yeast as a prophylactic; subacute scurvy promptly yielding to orange juice.

The second case was that of a baby who had been admitted to the institution when 16 days old, weighing 6½ pounds. It was fed on Schloss milk and weighed 9½ pounds when 3 months of age. From this time, however, it failed to gain, so that it was considered a favorable case for which to prescribe yeast. As will be noted (Chart 2), yeast was given for three weeks without effect. A change was then made to orange juice, with the remarkable result noted in the chart, namely, a prompt gain in weight, although the consumption of food remained the same. In addition there was an improvement in the general condition and a subsidence of eczema of the face, a phenomenon noted in two other cases following the administration of orange juice. We regard this last case as an instance of latent scurvy in an exceptionally young infant.

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[2] Schloss milk is composed of 140 c.c. of milk, 140 c.c. of 20 per cent. cream, 35 gm. of dextrimaltose, 15 gm. of flour, 5 gm. of plasmon, 0.2 gm. of potassium chlorate and 700 c.c. of water. The milk and cream are pasteurized, and added to the other ingredients after they have been boiled for fifteen minutes and cooled.

A third case is that of a baby who entered the institution when almost 6 weeks old, weighing 4¾ pounds. It did well on Schloss milk, and weighed 7¼ pounds at 3 months, and 8¾ at 4 months, when yeast was added to the diet. About two weeks later its weight curve began to flatten. When 5 months old it contracted nasal diphtheria and its appetite was impaired. This infection yielded to antitoxin and the appetite improved. However, no gain in weight could be effected, even though cereal was added to the diet. The child was pale, had marked eczema of the neck and back, rapid respirations, tachycardia, and again loss of appetite. Orange juice was given, and all the symptoms abated, including the eczema and the cardiorespiratory disturbance.

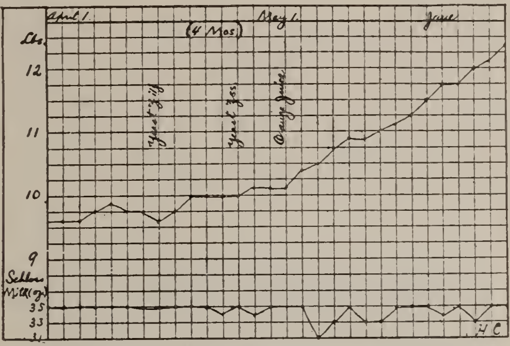

Chart 2.—Failure of yeast as a prophylactic; latent scurvy promptly yielding to orange juice.

In previous papers mention has been made of cases of mild scurvy developing on pasteurized milk. In two of these cases the autolyzed yeast was made use of in the attempt to effect a cure. The case illustrated in Chart 3 is one which was reported in detail in a recent paper treating of growth in scurvy[3] to illustrate the point that an infant who has been under-

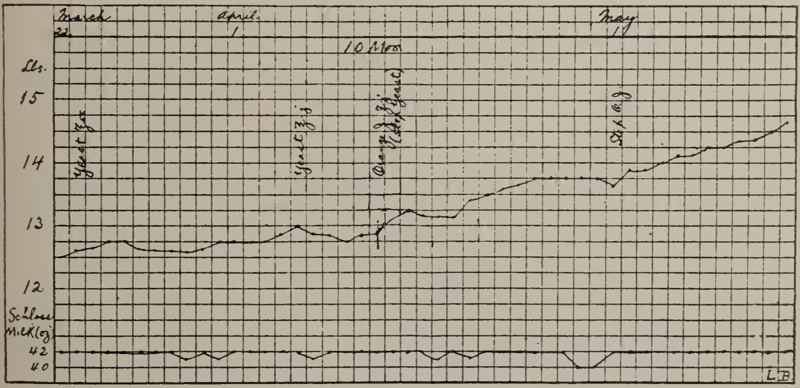

Chart 3.—Failure of yeast as a curative antiscorbutic.

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3. Hess, A. F.: Am. Jour. Dis. Children, 1916, 12, 152.

fed may develop scurvy, notwithstanding a satisfactory gain in weight. When this baby showed scorbutic signs, much to our surprise, as it had seemed to be progressing well, we attempted a cure by means of yeast. This was given for eighteen days, in daily quantity of one-half ounce and later one ounce. The peridental hemorrhages, the pallor, the lack of gain, the rapid respiration persisted, nevertheless. The hope of accomplishing a cure by this means was then abandoned and orange juice was substituted for the yeast. This resulted in a prompt disappearance of the hemorrhages and a coincident improvement in all the symptoms.

As regards the other case in which yeast was employed as a curative agent, little need be added to what is illustrated in Chart 8. It will be seen that in this case, that of a well-nourished infant, both egg yolk[4] and germ meal water were tried before the autolyzed yeast. Under the yeast therapy the appetite decreased rather than improved, the general symptoms and the periosteal tenderness became more marked, so that after a trial of four days we resorted to orange juice. This brought about a decided improvement within from twenty-four to forty-eight hours.

It will thus be seen that yeast failed signally as an antiscorbutic from a prophylactic as well as from a curative point of view. If it possesses this property at all, it is in too slight a degree to be recognized clinically, and negligible from a practical and therapeutic point of view. Although this result was discouraging and not what had been anticipated, it shows conclusively that however closely allied disorders such as beriberi and infantile scurvy may be, they must be regarded, in a clinical as well as in a chemical sense, as due to substances which are therapeutically totally different.

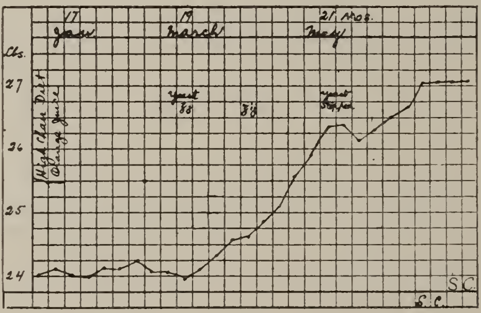

Chart 4.—Growth stimulated by means of autolyzed yeast in an older baby who had not gained for over two months.

Although yeast proved to possess little or no value as an antiscorbutic, it was found to be an excellent stimulant of growth. Thus the differentiation between an antiscorbutic and an essential growth stimulant—a distinction which was emphasized in the article in this series treating of growth in scurvy—was again substantiated. Following the addition of the yeast to the milk, there was in many instances a prompt rise in the weight curve. This sharp reaction led at first to the misconception that we were dealing with an excellent antiscorbutic substance. This inference was especially plausible in cases in which a gain was induced where none had taken place for weeks or months. But, as has been mentioned, the two phenomena were found to be by no means interdependent. In some instances infants gained 9 or 10 ounces the first week of yeast administration and then ceased to increase in weight; in others the gain was continued for several weeks. This favorable effect on growth was due, no doubt, in some cases to the stimulation of appetite, but in other cases there was a prompt response although the total intake of food remained the same. The yeast also had a favorable effect on the digestive processes, as shown not only by an increase of appetite, but by the character of the stools, which in some instances became normal where previously they had been constipated or poorly digested.

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[4] According to McCollum and Davis egg contains both the fat and water soluble accessories for growth, Jour. Biol. Chem., November, 1915, 23, 181.

After it had been found that autolyzed yeast stimulated the growth of infants, it was determined to attempt to stimulate the growth of some older babies, who had maintained an almost constant weight for a period of weeks and months, in spite of various changes of diet. These children were from 1½ to 2 years old, and received a diet of milk, cereal, vegetables and fruit, which is termed "high chair diet" in the institution. In some instances cod liver oil had been given without avail. Yeast was now added to the diet, either in the autolyzed form or as yeast powder, which is prepared by drying the compressed brewer's yeast. One-half ounce of the latter was added to the cereal or soup. It is unpalatable in whatever medium it is given, and although these children were successfully coaxed to take the prescribed amount, in many instances it was difficult to accomplish. Our results with these older infants were encouraging, as both the fluid and the dried yeast caused a definite gain in weight. This is well illustrated by Charts 4, 5 and 6, representing results in two patients who received fluid yeast and one who was given the desiccated preparation.

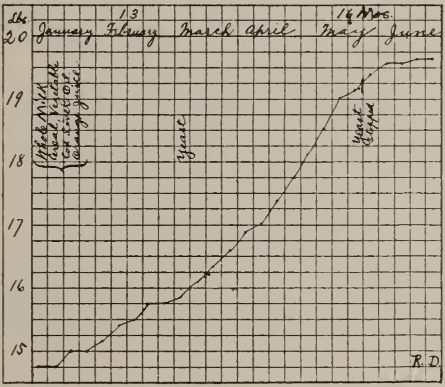

Chart 5.—Growth stimulated in an older baby by means of autolyzed yeast; gain of a pound in the two months previous to giving yeast, and of 2½ pounds in the subsequent two months.

In a previous paper treating more particularly of the relation of infantile scurvy and beriberi, mention was made of the use of wheat middlings as an antiscorbutic,[1] the subject being merely touched on and referred for detailed consideration to a later time. The wheat middlings were added to the wheat cereal (farina) in order to note its value as an antiscorbutic. In this connection a few words of explanation would seem in place. Farina is the starchy kernel of the wheat from which the outer layer has been removed. It has been repeatedly shown that beriberi is brought about by a diet of overmilled rice, which has been deprived of the outer layer or pericarp. It has likewise been established that the addition of rice polishings to the food suffices to effect a rapid cure of beriberi in man, or of its counterpart, polyneuritis, in birds. In view of these facts and the clinical similarity between infantile scurvy and beriberi, we decided to substitute in place of the farina a cereal containing the outer layer of the wheat. To this end we prepared a food consisting of two parts of middlings (the pericarp of the wheat) and one part of flour, and gave this in place of farina. No other change whatsoever was made in the diet. In some instances of scurvy improvement was noted; however, this could not be compared to the miraculous change brought about when orange juice was given. Frequently the gain in weight was not maintained, and in some cases there was not a concomitant improvement in the general symptoms characteristic of a mild form of scurvy. It seemed as if the middlings contained a small amount of the essential antiscorbutic substance, the vitamins of Funk, but a quantity insufficient to reestablish an adequate positive balance of this material. In view of the excellent results obtained by McCollum and his co-workers with the germ or embryo of wheat, it was determined to prepare a cereal containing this substance instead of middlings, to add this germ meal cereal to the dietary of pasteurized milk, and to observe whether it would not act as a substitute for orange juice. It will be remembered that McCollum and his co-workers found that they could produce marked growth in rats by adding wheat germ to the diet, and claimed that the germ contains two active accessory substances, one soluble in fat and the other soluble in water and stable to heat.[5] They also found that 8 per cent. wheat bran was no more efficient than 4 per cent. wheat embryo in promoting growth. More recently Sullivan and Voegtlin have reported the presence of an antineuritic substance in wheat bran, which may be obtained by means of hydrolysis and proper fractionization.[6]

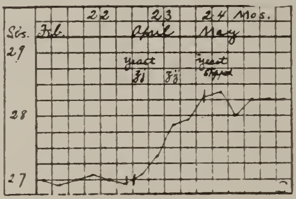

Chart 6.—Growth stimulated in an older baby by means of dried yeast; immobile weight for five weeks; gain of 1¼ pounds in the five weeks subsequent to giving yeast.

Wheat embryo is particularly rich in fats, the preparation which we employed containing 10.9 per cent. of ether-soluble extract. It was found that a cereal composed of equal parts of wheat germ and of farina was too rich for infants to take for long periods, and that they soon lost their appetites and refused it. If, however, only one-third of the germ meal was used, this loss of appetite did not ensue. Accordingly, a cereal was made of this composition, was cooked for two hours, as had been the custom, and was given twice a day in as large amounts as the individual infant would take, some consuming as much as eight or ten tablespoonfuls a day. The results may be summarized by the statement that this food

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5. McCollum, E. V., and Davis, M.: Jour. Biol. Chem., November, 1915, 23, 181.
6. Sullivan, M. X., and Voegtlin, C.: Jour. Biol. Chem., March, 1916.

was incapable of replacing orange juice and could not be relied on to prevent the development of subacute scurvy. The case represented in Chart 7 is illustrative of this point. Here we note an infant that received the germ meal when it was 6 months old. Notwithstanding this it showed definite signs of subacute scurvy, including tenderness and thickening of the lower end of the femur, three months later. This case is particularly interesting, as it illustrates the fact that an infant can gain in weight steadily while the scorbutic condition is slowly developing and advancing. In the last paper[3] an instance was cited, accompanied by a weight chart, of a baby previously underfed, who gained in weight for weeks and months while developing scurvy. The present case shows that these apparently paradoxical phenomena may be concurrent in a well-nourished infant of normal weight who has received a sufficient quantity of food. Of the six infants placed on the above cereal, three did satisfactorily, two showed some scorbutic signs, and a third appeared moderately well, but reacted later with a sharp gain in weight when orange juice was added to the diet, a reaction which we interpret as signifying that it had been receiving an insufficient amount of antiscorbutic foodstuff. On Chart 8 it will be observed that "germ meal water" is recorded. This is an extract prepared somewhat after the method of the barley water, which is so commonly used in infant feeding; one tablespoonful of wheat germ meal is added to a pint of water, allowed to soak for two hours, and then boiled for two hours. We obtain in this way the water soluble substance of the germ meal, which, according to McCollum, has a remarkable growth stimulating effect, and is responsible for the cure in polyneuritis.[7] It will be seen that in this case giving the extract of the germ in addition to the cereal failed either to improve the appetite or to bring about a gain in weight; it may be added that it also failed to relieve the typical scorbutic symptoms. In some infants the addition of this germ meal water to the diet effected a sharp gain in weight, which was not the case, however, when the failure to gain was part of a scorbutic condition. Under ordinary circumstances stationary weight is to be regarded as a general nutritional disturbance; on the other hand,

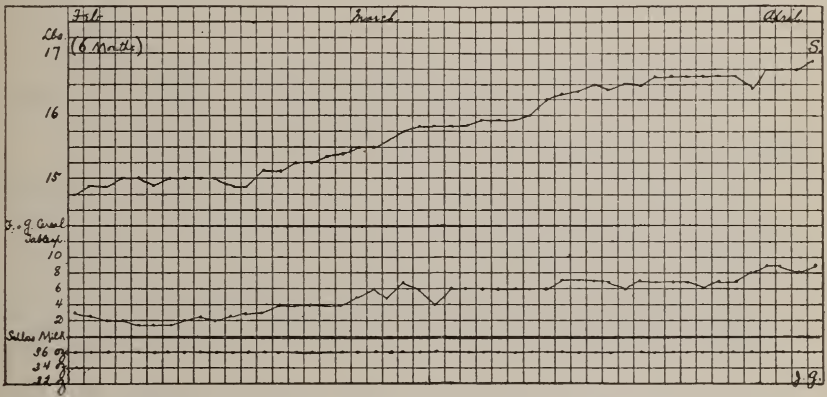

Chart 7.—Failure of wheat embryo as a prophylactic; development of subacute scurvy in a well-nourished infant despite gain in weight. (F. and G. cereal indicates farina and germ meal.)

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[7] McCollum, E. V., and Kennedy, C.: Jour. Biol. Chem., 1916, 24, 491.

when it is due to scurvy it must be considered specific in character and only to be remedied by specific therapy. In this respect the treatment resembles the specific serum therapy of the infectious diseases. In the above case (Chart 8) it will be noted that although orange juice also failed to bring about a gain, it immediately stimulated the appetite, which had reached a very low ebb; furthermore, it caused a marked improvement in the general condition and well-being of the infant. As the result of our own experience with wheat embryo and with middlings, we are of the opinion that both contain a certain amount of antiscorbutic substance, and that the embryo contains a decidedly greater amount. In neither of these parts of the wheat is specific vitamin present in a concentration comparable to that of orange juice. It may be contended that the amount of the embryo which we made use of was inadequate. In a sense this may be true. However, from a dietetic point of view it is approximately as large a quantity as can be fed to infants.[8] Although the embryo cannot serve as antiscorbutic, there is a decided advantage in the use of germ meal cereal as well as of germ meal water, as they contain a substance or substances which stimulate growth. There should be no misconception or confusion, however, as to their non-suitability, even in a prophylactic way, to replace orange juice. This is particularly to be regretted, as the preparation of a germ meal water which could be readily added to the milk instead of barley water would enable us to forego the necessity of giving our antiscorbutic food separately from the milk.

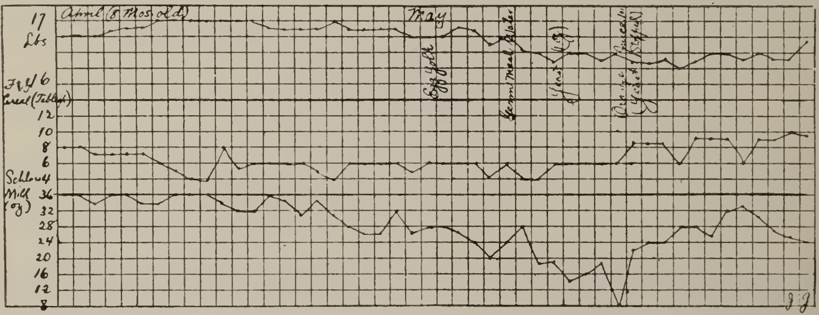

Chart 8.—Continuation of Chart 7; failure of egg yolk, germ meal extract and yeast as a curative antiscorbutic; improvement of appetite and general condition from orange juice.

Charts 7, 8 and 9 show the daily weight for a period of six months of an infant who developed subacute scurvy, presenting respectively the prescorbutic, the scorbutic and the reparative stages. In Chart 8 it will be seen that although orange juice increased the appetite, it could not bring about a gain in weight even after it had been taken for two weeks. The chart shows, furthermore, that when orange juice was discontinued and replaced by a tablespoonful of mashed potato (boiled), a steady increase in weight set in, accompanied by a marked stimulation of the appetite, more especially for the fluid food. Accompanying this change there was a decided improvement in the general condition. A reaction of this kind raises the question, not merely as to whether one of these antiscorbutics is the more potent, but as to possible qualitative differences among them, and whether one may not be better suited to the needs of one child than to those of another. This is a question to which, as may be imagined, it is at present impossible to give a direct answer.

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[8] Each tablespoonful of the cereal contained 1 gm. of dried embryo, so that the average infant took about 8 gm. a day in this form. Each quart of Schloss milk contained about 21 gm. of the watery extract. It will thus be seen that the infant depicted in Chart 8 received from 10 to 15 gm. of this active substance in addition to the cereal.

It is evident, however, that in this particular case one tablespoonful of potato was clearly more potent than one or even two tablespoonfuls of orange juice. In the paper by Hess and Fish[9] two mild cases of scurvy were mentioned, which developed in spite of a diet including vegetables. Reports of other observers would also lead to the conclusion that some infants are unable to utilize thoroughly certain well-established antiscorbutics. There are, for example, undoubted cases scattered through the literature which developed scurvy despite the use of orange juice, just as there are others in which such symptoms have manifested themselves notwithstanding a diet of raw cow's milk, or even, most rare of all, of breast milk. Finkelstein's[10] well-known experience of infantile scurvy developing in two successive children of a family, although prophylactic measures were employed to avoid the occurrence in the second infant, is a striking example of the fact that a definite metabolic disturbance of this kind may exist in certain persons and families, which cannot be counterbalanced by specific therapy. It is conceivable that orange juice, potato, green vegetables and other antiscorbutic articles of diet do not supply entirely the same essential substances; that these substances differ slightly in their chemical constitution, or that one food may contain more than a single substance of this nature. These reasons may account for the facts that in some cases of subacute scurvy, although we meet with a prompt reaction to orange juice, we find that this good effect is enhanced by substituting potato, a result which has been witnessed several times, or that the beneficial effect of orange juice is still further intensified when a vegetable is added to the dietary.

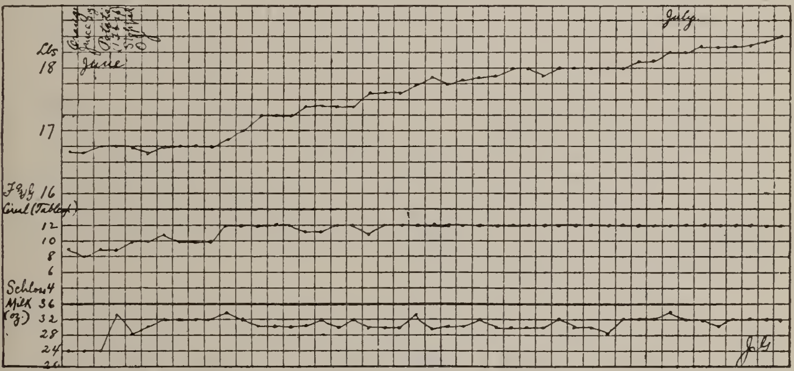

Chart 9.—Continuation of Chart 8; superior antiscorbutic effect of potato, in increasing appetite, in causing gain in weight and in improving the general condition.

A question perhaps of more theoretical than practical interest is that of the power of the body to store the vitamins. It is generally believed, and seems the most plausible explanation, that infants do not develop scurvy during the first few months of life on account of the fund of antiscorbutic material which they have inherited from their mothers and brought with them into the world. If in postnatal life these substances are not supplied, a negative balance ensues and infantile scurvy gradually results. An observation which we were in a position to make leads us to believe that the tissues have a remarkable power for storing antiscorbutic vitamins. The case is the one previously reported: an infant who had been markedly underfed before entering the institution and who gained for some months after admission, but then showed signs of subacute scurvy, such as periosteal tenderness, pallor, peridental hemorrhage, and tachycardia. One ounce of orange juice was given for a period of sixteen days, in other words, a pint in all. By this time the scorbutic symptoms were relieved, the hemorrhages into the gums were no longer to be seen, and the general condition was greatly improved; accordingly, the antiscorbutic diet was discontinued. This was on the first of May. The infant weighed almost 14 pounds at this time, and was nearly 11 months old. During May he gained three-quarters of a pound. Farina was then added to his diet, besides the 42 ounces of Schloss milk. In June he gained about 1½ pounds. He gained nothing in July, but showed no signs of scurvy. In August 1 ounce of orange was again given daily. Although no greater amount of food was consumed, there was at once an increase in weight, a gain of 12 ounces during the first week and an improvement in the general condition. We interpret these phenomena as indicating that the sixteen ounces of orange juice had protected the child for a period of two months and that he then lapsed into a condition of latent scurvy. A negative balance of vitamins was evident from the child's nutritional stagnation, considered in conjunction with the prompt growth reaction on the administration of orange juice.

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[9] Hess, A. F., and Fish, M.: Am. Jour. Dis. Child., 1914, 8, 385.

[10] Finkelstein, H.: Lehrbuch der Säuglingskrankheit, 1912, 2, 480.

Conclusions.

As it had been shown that a clinical similarity exists between beriberi and infantile scurvy, it seemed as if yeast, which is a specific in the former disease, might also prove to be a potent antiscorbutic. Clinical experience did not substantiate the analogy, but on the contrary demonstrated that this substance is of no value in scurvy either as a prophylactic or as a curative dietetic agent. It was found, however, to possess virtue as a stimulant to growth, when added to the milk in the form of the fluid autolyzed yeast or given to older infants as a desiccated powder.

The embryo or germ of the wheat seed is another effective therapeutic agent in the cure of beriberi or in avian polyneuritis. Accordingly, this was also tested clinically for its antiscorbutic power; for this purpose a cereal and a watery extract of the germ meal were prepared. These preparations manifested some value as antiscorbutics, but not sufficient to render them useful in this connection from a practical or clinical standpoint. The watery extract, which is readily prepared, was found to stimulate growth, and may be used to advantage, on account of this quality, as a substitute for barley in milk preparations.

It was furthermore noted that there is an idiosyncracy as to the reaction of the individual to well-established antiscorbutics; for example, in some instances orange juice may meet with only partial success while potato produces a rapid cure.

PROVOCATIVE AND PROPHYLACTIC VACCINATION IN VAGINITIS OF INFANTS*

ALFRED F. HESS.

We have had to contend with the problem of vaginitis, just as so many others have had to do in similar institutions. It is not my intention to write a review of the trials and tribulations encountered during the five years since we have been housed in modern buildings and have made every effort to combat this insidious disease. As we have profited in some respects by experience, however, it seemed as if it might be of value to those who are actively interested in this problem to communicate briefly some of the lessons learned during this period.

Our endeavors have been variously directed: in preventing the admission of infected infants, in attempting in many different ways to avoid a spread of infection, in diagnosing cases at the earliest possible moment, and, finally, in resorting to every means to effect a cure. It should be realized at the outset that, although the distinction is not always sharply drawn, vaginitis presents a problem in a home or asylum for infants totally different from that which it presents in a hospital. In the latter the solution is comparatively simple, for all that is necessary in order to eradicate the disease is to cease admitting girl infants and to discharge those infected, one by one, as they are cured of the ailment for which they were admitted to the hospital. In the asylum, on the other hand, when a case of vaginitis slips past the admitting physician, or arises apparently de novo in one of its wards, it is realized that a heavy burden has fallen on the medical staff, for this infant will have to be guarded under quarantine for months or years, and will at all times constitute a threatening source of infection. We have had a variable amount of vaginitis; at times a lull, at others a sudden increase in the number of patients. At present, due to the regulations which are in force, the number of cases is comparatively small. It may be remarked, however, that during these years no instance of ophthalmia, of arthritis, or of infection of any of the personnel has occurred. There have been four instances of specific urethritis in boy infants, and one of rectal infection. This urethritis lasted but a few weeks and did not give rise to a positive complement fixation reaction, so that we believe it involved merely the anterior urethra.

The diagnosis of gonococcus vaginitis is not always easy to establish. In the first place, it is at times confused with other types of this disease, for there is no doubt that such an inflammation may be due to micro-organisms other than the gonococcus. For months we harbored a case due to the streptococcus which was present in almost pure culture in the vaginal discharge and was furthermore identified in cultures taken from the cervical canal at postmortem examination. There was an absence of gonococci both during life and after death. Such cases as this are not, however, the difficult instances we refer to, but the borderline cases which are exceedingly puzzling, especially those showing merely pus cells on microscopic examination. If these cells are numerous, an inflammation is undoubtedly present, and in the great majority of cases the infecting organism will prove to be the gonococcus. The specific nature of this infection is all the more probable if there are no micro-organisms to be seen in the field among the cells. One exception should be borne in mind as to the diagnostic significance of pus cells. This subject was called to our attention by noting many leukocytes in the smear of an infant only 48 hours old, who was brought to the institution for admission. It hardly seemed as if this were an instance of gonococcus vaginitis, although it is perfectly possible for an infant to be infected in this way during parturition, so we had some tests carried out in order to ascertain how often pus cells are encountered in smears taken from infants during the first two days of life. These tests were kindly carried out by Dr. Edwin Langrock. They showed that in about 50 per cent. of infants pus cells may be found in vaginal smears taken within the first forty-eight hours of life, so that it would seem that they must not be regarded as pathologic, but probably as the reaction of the external tissues to the inevitable invasion by bacteria after birth. Possibly a similar inflammatory reaction occurs in the intestinal canal.[1] It should be understood that for these vaginal smears, as well as for all others referred to in this paper, an applicator tipped with moistened cotton was inserted deep into the vagina, so as to come in contact with the cervix.

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* Published in Am. Jour. Dis. Children, 1916, 12, p. 466.

As we inquire deeper into the cause of the spread of vaginitis, it would seem to resolve itself, in the last analysis, to a consideration of but one or more phase of the general problem of the dangerous but healthy carrier. Although there can be no doubt that the disease is frequently communicated by means of clothing or utensils, the fundamental cause of the infection of one or more infants, in institutions where every care is exercised, must be considered the latent carrier, some healthy infant who harbors the gonococcus. Such has been our experience. For two years a pathologist devoted about two hours daily to the making of routine microscopic examinations of vaginal smears of the infants in the asylum. Wherever a case of vaginitis arose, the routine procedure was to make examinations three times during the following week on every infant in the ward in order to ferret out the source of the infection. A card catalogue was kept both of the individual infants and the various wards. Repeated tests brought to light some case in which, in spite of the absence of discharge, gonococci were evident in the smears. Recrudescences of infection occurred in one ward or another every few months and sometimes oftener. We are confronted, therefore, with a problem similar to that in typhoid or diphtheria, in which the carrier constitutes the main stumbling block in the path of prophylaxis. In the case of vaginitis the solution is rendered still more difficult in view of the heightened susceptibility of infants to infection.

During the past five years necropsies have been performed on four infants who had vaginitis while in the institution. (We are able to carry out postmortem examinations on fully 75 per cent. of the children who die.) This offered an exceptional opportunity to gain an insight into the pathologic changes of the common nonvirulent form of this infection. The pathology of this disease is generally glossed over in textbook descriptions. Furthermore, judging from the literature, we conclude the number of postmortem examinations of cases of this type is not large. We should expect this to be the case, as very few hospitals admit vaginitis, which must therefore constitute merely a chance pathologic condition met with in the course of some primary fatal disease. One of our cases had existed but three weeks, two patients were known to have had vaginitis for months, and one for a year or more. They all showed the same pathologic condition, which may be summarized in a few lines: Macroscopically the vagina appeared negative, as did the body of the uterus and the appendages. The only abnormal condition was redness of the tip of the cervix, which, however, did not extend along the canal to the internal os. Microscopic examination confirmed the gross anatomic appearance of these structures. In every instance the entire vagina, the uterus and the tubes were removed for microscopic examination, which was kindly carried out by Drs. Eli Moschowitz and Alwin Pappenheimer, whom I take pleasure in thanking for their courtesy in this connection. The sole lesion found was an inflammation of the cervix about the external os with a round-cell infiltration of the submucous tissues. Guided by these postmortem examinations, we must regard, it would seem, the average gonococcus infection as involving the cervix rather than the vagina, and must consider the infection a cervicitis rather than a vaginitis. It is in the cervix that the gonococcus finds a most favorable nidus. In a study published a few years ago from this service by Rubin and Leopold,[2] an inflammation of the cervix was described as seen frequently by direct examination. In the recent preliminary report of the vaginitis committee of the American Pediatric Society[3] it may be noted that eleven gynecologists who responded to their questionnaire thought that the cervix or uterus at times showed evidences of this disease. In the adults it has been reported that 95 per cent. of the chronic cases showed a cervicitis.[4] As the result of personal experience we must conclude that the vagina is comparatively immune in this infection, especially when the disease has reached the subacute or chronic stage.

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[1] In this connection it would seem of interest to note that leukocyte counts of new-born infants were found frequently to be high, the total per cubic centimeter reaching from 15,000 to 23,000, with a differential cell count presenting the adult type, namely, from 60 to 75 per cent. of polymorphonuclears.

There seems to exist a very general impression that vaginitis is more particularly a disease associated with child-caring institutions, and that infants brought up in the homes of the poor are infected far less frequently. This has not been our experience. During the year 1915 sixty-six infant girls were admitted to the institution and seventy-two rejected; of these seventy-two, sixty were refused admission on account of vaginitis, thus making the total infection noted in these children almost 50 per cent. It should be added that only three of the rejected patients were referred from other institutions, and that the remainder came direct from their homes. Further investigation may possibly show that this ratio of positive cases is exceptionally high. As a result of having to refuse so many infant girls the institution now harbors one-third girls and two-thirds boys. It would be of theoretical as well as of great practical interest if we could know the results of a similar examination of older girls. From a limited experience of girls between the ages of 4 and 10 at the Tuberculosis Preventorium, I may state that not a few children of this age show signs of infection, although the danger of the spread of the disease seems to have become greatly lessened at this age.

One of the great difficulties in trying to combat vaginitis or cervicitis is that we find it impossible to recognize the infectious case in its earliest stage, in other words, the danger of the latent carrier. To overcome this difficulty we have made many attempts to convert the carrier into an open or florid case. Various drugs have been injected into the vagina in order to bring this about, but as they were found of no practical value, it would serve no purpose to enter into detail concerning efforts of this kind. For the past year, with this end in view, all infant girls have received three subcutaneous injections of gonococcus vaccine soon after they were admitted to the institution. These infants, it should be understood, had all shown an absence of pus cells on admission. The vaccine was prepared from a culture isolated from one of the cases in the institution, and 250, 500, and 750 millions were injected with three day intervals. The object of these vaccinations was to determine whether they would prove provocative and bring to light latent infections. In adults with urethritis it has been found by Asch[5] that injections of vaccine lead to a discharge containing pus cells and gonococci. The dosage which we used was entirely empirical. As a result of experience it would seem that it could be much smaller; indeed, at the present time we are giving only 100, 200 and 400 millions. Moreover, two injections may prove sufficient, as we have rarely brought about a discharge by means of a third inoculation. During the past year such provocative injections have led to the discovery of eight new cases during the first week or two following their admission (Table I), and to the unprecedented result that not one case of vaginitis has slipped into the main institution from the admitting pavilion. We have made use of this diagnostic aid not only at the time of admission, but in order to discover carriers in dormitories, where sometimes sporadic cases of vaginitis arose (Table 2). For example, in one ward of twelve infants in which the girls had been free from vaginitis for a period of six to eighteen months, an active case suddenly developed, and although cervical smears failed to disclose its source, two carriers were brought to light by means of provocative inoculations. This has been the case on other occasions. This spring one case of vaginitis with gonococci and another showing pus cells, but no micro-organisms, were unexpectedly and unaccountably noted in the infirmary. There were six infant girls at the time in this ward. Provocative inoculation disclosed four positive cases after a second inoculation. Here was a veritable latent epidemic, which was uncloaked by means of the vaccine.

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[2] Rubin, I. C., and Leopold, J. S.: Am. Jour. Dis. Child., 1913, v, 58.
[3] Tr. Am. Ped. Soc., 1915, xxvii, 331.
[4] Menge, K.: Handbuch der Geschlechtskunde, Vienna, 1912, ii, 323.

We are unable to state the exact scientific basis of the reaction following these inoculations.. It is, however, not due to a rise of temperature. This was evident, as it occurred to a marked extent in some instances in which there was no febrile reaction whatsoever. It cannot be regarded as absolutely specific, for a positive result was brought about at times by similar injections of staphylococcus vaccine, although this was not found to be so reliable for this purpose as that made from the gonococcus. Indeed it is becoming more and more evident that many similar reactions of the tissues which we have been wont to regard as specific in nature, must be considered in large measure systemic cellular reactions. Whatever may prove to be its exact scientific basis, we firmly believe that the reaction to gonococcus vaccine will be found of great practical aid in combating this difficult infection. The same principle might perhaps be applied to facilitate the recognition of carriers in other infections, for example, typhoid fever and pyelitis.

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[5] Asch, P.: München, med Wchnschr., 1915, No. 39.

TABLE 1.

ROUTINE PROVOCATIVE VACCINATIONS.*

Name.	Age.	Admitted.	Date of Vaccination.	Cervical Smear.	Vaginal Discharge.	Macroscopic Pus.	Microscopic Pus.	Gonococci.	Remarks.
M. G.	8 mo.	11/15/14	....	11/15/14	—	—	—	—	
				12/12/14	—	+	+	—	
			4/27/15	4/25/15	+	+	+	—	
				5/ 1/15	+	+	+++	+	
				5/ 3/15	+	+	+++	+	
A. S.	11 mo.	4/15/15	....	4/15/15	—	—	—	—	
				4/20/15	—	—	—	—	
			4/24/15	5/ 6/15	+	+	+	+	Smear after third injection loaded with gonococci; discovered in reception pavilion.
				6/ 1/15	+	+	+	+	
				7/10/15	+	+	+	+	
				9/ 8/15	—	+	++	+	
H. B.	3 mo.	12/21/15	....	12/21/15	—	—	—	—	
			2/ 2/16	2/ 9/16	—	—	—	—	Vaccine given a second time to discover latent infection in this ward.
				2/25/16	—	—	++	—	
			3/ 1/16	3/16/16	—	+	++	—	
				3/29/16	—	+	++	+	
R. G.	11 mo.	12/14/14	....	12/14/14	—	—	—	—	
				1/ 9/15	—	+	—	—	
				3/ 1/15	—	+	++	—	
			4/27/15	5/ 5/15	+	+	+	+	Vaccinated in order to find gonococci; a discharge resulted which lasted about a month and a half.
				6/ 1/15	+	+	+	+	
E. S.	3 yr.	11/ 1/15	....	11/ 1/15	—	—	—	—	
			11/21/15	11/29/15	+	+	+	+	Vaginal discharge incited, which lasted about three weeks.
				12/16/15	+	+	+	+	
				3/ 7/16	—	—	—	—	
				3/10/16	—	+	+	+	
				4/11/16	—	+	+	—	
M. H.	3 yr.	7/13/15	....	7/13/15	—	—	—	—	
			7/15/15	7/29/15	+	+	+	+	Vaginal discharge incited, which lasted about two weeks; discovered in reception pavilion.
				8/ 3/15	—	±	+	+	
				8/ 9/15	—	—	—	—	
				3/10/16	—	+	+	—	
				4/14/16	—	—	+		

*Date of vaccination is always that of the first injection; two other injections were given at three day intervals.

TABLE 2.
PROVOCATIVE VACCINATION IN OLD CASES OF VAGINITIS, LATENT CARRIERS.*

Name.	Date of Vaccination.	Cervical Smear.	Vaginal Discharge.	Macroscopic Pus.	Microscopic Pus.	Gonococci.	Remarks.
S. L.	....	1/15/14	+	+	+	—	
		12/20/15	—	—	—	—	
		12/30/15	—	—	—	—	
	2/ 1/16	2/ 6/16	—	—	+++	—	Positive result after negative period of two years.
		2/ 9/16	—	—	++	+	
		4/20/16	—	—	+	—	
F. G.	....	11/ 8/14	—	+	—	—	
		12/20/14	—	+	+	—	
		12/20/15	—	—	+	—	
		12/30/15	—	—	—	—	
	2/ 1/16	2/ 6/16	—	—	+	—	No temperature reaction.
		2/ 9/16	—	—	+	—	
		4/20/16	—	—	+++	++	
		5/ 7/16	—	—	+	—	
L. E.	....	11/ 8/14	—	+	+	—	
		12/20/14	—	+	++	—	
		12/20/15	—	—	+	—	
		12/30/15	—	—	+	—	
	2/ 1/16	2/ 6/16	—	—	++	—	No temperature reaction.
		2/ 9/16	—	—	+++	+	
		4/20/16	—	—	—	—	
G. S.	....	11/10/14	—	+	+	+	
		12/ 8/14	—	+	++	—	
		1/20/15	—	+	+	—	
		12/15/15	—	—	—	—	
		12/30/15	—	—	—	—	
	2/ 1/16	2/ 6/16	—	—	+++	+	
		2/ 9/16	—	—	++	+	
		4/20/16	—	—	—	—	
F. G.	....	6/ 8/14	+	+	+	+	
		12/15/14	—	+	+	—	
		1/12/15	+	+	+	—	
		12/20/15	—	—	—	—	
		12/30/15	—	—	++	—	
	2/ 1/16	2/ 6/16	—	—	++	—	
		2/ 9/16	—	—	++	+	
		4/20/16	—	—	+	+	
R. L.	....	5/18/14	—	+	++	—	
		12/20/14	—	+	+	—	
		12/18/15	—	—	—	—	
		12/30/15	—	—	—	—	
	2/ 1/16	2/ 8/16	—	—	++	—	
		2/ 9/16	—	—	++	+	Went home in April.
S. R.	....	10/12/14	—	+	—	—	
		12/15/14	—	+	+++	—	
		1/18/15	+	+	+	—	
		12/20/15	—	—	—	—	
		12/30/15	—	—	+	—	
	2/ 1/16	2/ 6/16	—	—	++	—	
		2/ 9/16	—	—	++	+	
		4/20/16	—	—	++	+	
B. K.	....	12/10/14	—	+	—	—	
		1/12/15	—	+	—	—	
		12/20/15	—	—	—	—	
		12/30/15	—	—	+	—	
	2/ 1/16	2/ 6/16	—	—	+	—	
		2/ 7/16	—	—	++	+	
		4/30/16	—	—	+	—	

*See footnote, Table 1.

The vaccine was found to be of value not only as a diagnostic, but, to a certain extent, as a prophylactic measure. To this end we used it on about seventy-five infants and were able to change the entire nature of vaginitis in our institution. In patients who were vaccinated the vaginitis showed itself as a mild type of infection. It is not to be expected that prophylactic injections can prevent the occurrence of carriers; neither can diphtheria antitoxin obviate the occurrence of diphtheria carriers nor typhoid vaccine prevent the implantation of typhoid bacilli in the mucous membrane. However, the protected patients, instead of developing a vaginal discharge full of pus cells and gonococci, were found to have no discharge whatsoever, and to show as the only evidence of infection a few pus cells and micro-organisms in the cervical smears. In other words, a nonclinical type of disease resulted. The bacteria appear atypical frequently under these conditions; they are degenerated and often very small, as has been described by many in connection with chronic cases. It is too early to state how long this partial immunity will last. We have not attempted to control its course by means of complement fixation, as our experience with this reaction has not been entirely satisfactory. For three years we made serum tests of this kind on the infants in the institution, in order to judge of the persistence of infection, but the results proved so contradictory that the attempt to solve the question by this means was abandoned.

There are some diseases which occasion not only a recrudescence of vaginitis, but seem to confer a susceptibility to infection. This is especially true of scarlet fever. In this disease the susceptibility to gonorrhoea extends still farther in that a systemic lack of resistance to the gonococcus results, so that joint infections and other evidences of a specific bacterial invasion result. Recently Nicoll[6] reported three cases of gonococcus arthritis in children from the scarlet fever pavilion of the Willard Parker Hospital. and others have been noted in these wards from time to time. However, there is found not merely an acquired susceptibility and probably also a natural susceptibility to gonococcus infection, but also in other cases a well defined natural immunity. This has been very evident in individual instances among the infants in the asylum.

At times it has been very difficult to carry out an absolute isolation of vaginitis patients, more especially when some infectious disease has visited the institution. For example, about two years ago, during a considerable epidemic of whooping cough, it became necessary to isolate the children according to the presence of pertussis rather than according to vaginitis. Under such conditions it was observed that certain infants did not develop vaginitis in spite of some months of exposure in these wards. In other words, these babies possessed a high degree of immunity (Table 3). Since then this idiosyncracy has been confirmed by further observations and there are at present five, or possibly six, infants in the institution who may be said to be immune, or almost immune, to this infection. During the past two years they have chanced several times to be in wards in which vaginitis broke out but nevertheless have escaped infection each time. These cases have been given provocative vaccination in order to make certain of their freedom from infection, but in every instance this has failed to incite any inflammatory reaction. We do not know whether this is to be considered a local or a systemic immunity. We may, however, call attention to the fact that there seems to be no doubt that a similar immunity exists in the adult. It is to be remembered in this connection that there is frequently a difference in the configuration of the epithelium in different individuals. In some, the columnar epithelium begins outside of the cervix, in others the squamous epithelium, which is typical of the vagina, extends even into the cervical canal. These variations, as well as others relating to the number, the size, and the patency of the cervical glands, have been shown to be present in earliest infancy.[7] As is well known, the gonococcus has a particular affinity for surfaces covered with columnar epithelium, and is not able to attack those lined with squamous cells. The immunity described above is rare. In many instances it is probably not absolute and sooner or later, in the course of months, it will be found that infection takes place. It is reported as an observation of interest, rather than as one which can play a role in the epidemiology of this disease.

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[6] Nicoll, M.: Arch. Pediat., 1914, No. 11, 804.

TABLE 3.

CASES OF NATURAL IMMUNITY.*

Name.	Admitted.	Age on Admission.	Cervical Smear.	Vaginal Discharge.	Macroscopic Pus.	Microscopic Pus.	Gonococci.	Provocative Vaccination.
P. K.	5/29/12	6 mo.	6/24/14	—	+	—	—	
			8/16/14	—	—	—	—	
			9/15/14	—	+	—	—	
			12/30/14	—	+	—	—	
			7/ 8/15	—	—	—	—	
			7/15/15	—	—	—	—	7/15/15
			7/20/15	—	—	—	—	
			3/ 7/16	—	—	—	—	
			5/ 4/16	—	—	—	—	
R. C.	5/23/13	2 yr.	5/24/14	—	+	—	—	
			5/26/14	—	—	—	—	
			6/24/14	—	+	+	—	
			1/ 5/15	—	—	—	—	
			5/14/15	—	+	—	—	
			7/ 9/15	—	+	+	—	7/15/15
			7/20/15	—	+	+	—	
			9/15/15	—	—	+	—	
			12/30/15	—	+	—	—	
			2/24/16	—	+	—	—	
			3/ 6/16	—	—	—	—	
N. S.	4/21/14	3 yr.	7/25/14	—	—	—	—	
			7/ 8/15	—	—	—	—	
			7/10/15	—	—	—	—	
			7/20/15	—	—	—	—	7/15/15
			9/ 7/15	—	—	+	—	
			12/30/15	—	+	—	—	
			2/ 6/16	—	+	++	—	
			3/ 4/16	—	—	+	—	
			5/ 4/16	—	—	+	—	
F. B.	6/16/12	2 yr.	6/24/14	—	+	—	—	
			7/10/15	—	—	—	—	7/15/15
			7/20/15	—	—	—	—	
			9/ 7/15	—	—	+	—	
			12/30/15	—	+	+	—	
			3/ 4/16	—	—	—	—	

* Unavoidably exposed for some months during spring of 1914, due to pertussis epidemic.

CONCLUSIONS.

Postmortem examinations show that in the subacute and chronic cases of vaginitis in infants the cervix is most frequently involved and that the vagina generally shows no signs of inflammation. Cervicitis would therefore seem to be a more correct term, in this connection, than vaginitis.

Where numerous pus cells without bacteria are found in smears made from the cervix, an inflammation may be assumed to be present, and in the overwhelming majority of instances the inciting factor will be found to be the gonococcus. Other micro-organisms may, however, be the cause of the inflammatory process, for example a streptococcus, as in a case which was studied both during life and after death. It should be borne in mind that smears taken from new-born infants very frequently show pus cells, due probably to the invasion of the vagina by saprophytic bacteria and that, in the new-born, these cells should not be considered pathologic or as evidence of gonococcal inflammation.

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[7] Moericke, R.: Ztschr. f. Geburtsh. u. Gynäk., 1882, vii, 84.

Gonorrheal vaginitis, or cervicitis, should not be regarded as a disease encountered especially in institutions as it may be found in a considerable proportion of infants living in the crowded tenements in the city.

In child-caring institutions the greatest obstacle to limiting and controlling the spread of this disease is the difficulty of recognizing latent cases. The situation affords, therefore, but one more aspect of the problem of the healthy but dangerous carrier and of the difficulty of devising methods to prevent contact infection. By means of provocative inoculations of gonococcus vaccine we have found it possible to convert the concealed carrier into an open case, and in this way to discover many cases which had eluded detection. Vaccinations have also some prophylactic value, and may either confer protection or render subsequent infection of a mild character, so that it assumes a bacteriologic rather than a clinical type.

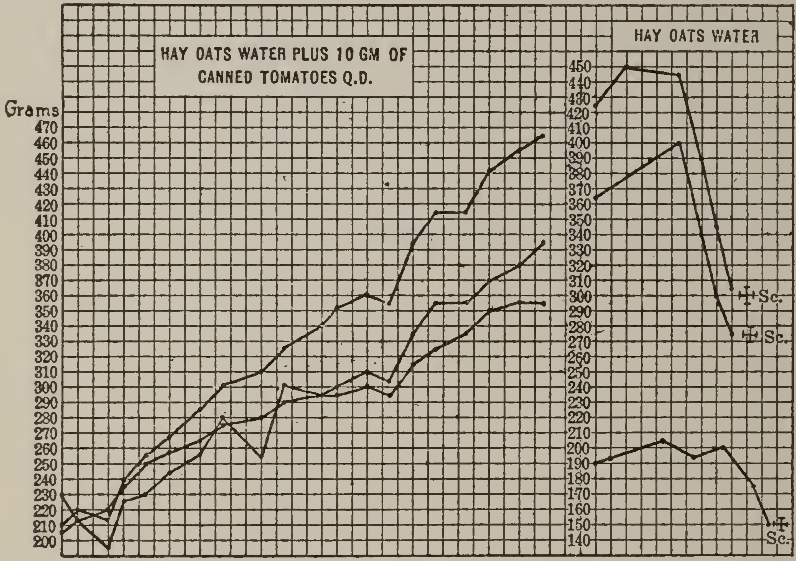
There is found not only a natural susceptibility to this infection and also an acquired susceptibility, such as occurs in the course of scarlet fever, but in some cases a natural immunity which may be sufficient to protect infants who come in contact with infected patients.

CANNED TOMATOES AS AN ANTISCORBUTIC.*

Alfred F. Hess and Lester J. Unger.

In view of the fact that canned tomatoes are included in our army ration, in which they may be substituted for potatoes to the extent of 20 per cent., it seems worth while to ascertain whether they possess antiscorbutic properties. This appeared particularly desirable in view of our previous experiments[1] which had demonstrated that the dehydrated vegetables commonly employed cannot be relied upon for furnishing this important dietary factor. From a theoretical standpoint a study of this question was of further interest, as the tomatoes have been subjected to a temperature above the boiling-point in the course of the canning process.

Various series of guinea pigs, five in each group, were put on a diet of hay, oats and water ad libitum and fed various amounts of strained tomatoes which had been canned almost one year previously. It was found that the addition of 5 c.c. of these tomatoes was sufficient to protect the animals from scurvy, and that larger amounts stimulated growth to a remarkable degree.



The three guinea pigs represented in the graphs on the left received canned tomatoes and thrived. The three on the right were not fed tomatoes and died of scurvy.

In view of this favorable experience on animals, for the past three or four months we have fed canned tomatoes to infants who were receiving pasteurized milk, substituting it in the dietary for orange juice which has become increasingly expensive. The amount given to babies three months or more of age was 15 c.c.; half this quantity was given daily to younger infants. The tomatoes have been uniformly well tolerated throughout the summer by babies as young as one or two months of age, and we can recommend this foodstuff as an economical and efficient antiscorbutic.

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* Published in Proc. Soc. for Exp. Biol. and Med., 1918, 15, p. 96.

[1] Hess, Alfred F., and Unger, Lester J., Proc. Soc. Exp. Biol. and Med., 1918, XV, p. 141.

A PROTECTIVE THERAPY FOR VARICELLA, AND A CONSIDERATION OF ITS PATHOGENESIS*

ALERED S. HESS and LESTER J. UNGER.

As is well known, chickenpox is one of the very mildest of the infectious diseases to which children are subject. It possesses, however, considerable medical interest as one of the group of diseases of unknown origin and because of its remarkable communicability. In the latter particular it is rivalled only by measles. In the Home for Hebrew Infants it has been our experience that in the course of an epidemic about three-quarters of the younger children develop varicella and about one-half of those above two years of age. It spreads rapidly through the institution, progressing from ward to ward in an uncontrollable manner, frequently necessitating quarantine for a period of many months. Among children in private homes it possesses a serious pedagogic aspect, often disrupting the school sessions for long periods because of its widespread prevalence and long incubation period, which causes the infection to drag along throughout the entire winter term. Therefore, when Kling[1] in 1913 reported that he was able to immunize children against this mild but exceedingly disturbing infectious disease by means of a single vaccination, we welcomed the announcement and decided to make use of it at the earliest opportunity. Kling's method consisted merely of applying the contents of the vesicles to the abraded surface of the skin after the manner in which a von Pirquet tuberculin reaction is carried out. Two years later a small epidemic of chickenpox made its appearance in our institution and Kling's method was put into practice. The result may be summarized by the statement that it afforded a certain degree of immunity, but that it was by no means satisfactory. This, we believe, has been the experience of others who have used this procedure. It seemed to point the way, however, to better methods. It was evident, from the partial success, that the virus was contained in the vesicles and it seemed probable that failure was due to a faulty and uncertain method of introduction.

During the past year varicella has been widespread throughout New York City and made its appearance in our admitting pavilions. An apportunity was therefore afforded us again to attempt immunization. To this end we made use of the contents of the vesicles, but instead of applying this fluid to the surface of the skin, we injected it intravenously. The technic was as follows:

> In each instance only vesicles and not pustules were made use of; the surfaces were very gently washed with sterile salt solution and the contents allowed to ascend into capillary tubes. As much as possible of the clear fluid was obtained and was immediately mixed with sterile normal salt solution. This diluted virus was diluted still further with salt solution before it was injected. The dosage was entirely empirical; ¾ inch of fluid, as measured in the capillary tube, was used in each instance.[2]

In all, thirty-eight children, about three or four years of age, were vaccinated intravenously after this method. None of the children developed any local or general signs of any eruption suggestive of varicella following the injection. They were all, in the course of the epidemic, unavoidably in contact with one or more cases of chickenpox, but in spite of this proximity only one developed the disease; this one thirty-six days after the date of inoculation.

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* Published in Am. Jour. Dis. Children, 1918, 16, p. 34.

[1] Kling, C.: Berl. klin. Wchnschr., 1913, 50, 2083.

[2] All children in the institution who have a large spleen, bilateral epitrochlear glands or give a family history of insanity, are subjected to the Wassermann test, so that all danger of syphilis was guarded against.

It is evident, therefore, that by means of these intravenous vaccinations almost absolute immunity was brought about with no symptoms of the disease developing as the result of the attempted immunization. This result, paradoxical as it may at first appear, may be interpreted as follows: It would seem that most viruses are able to bring about their specific diseases only when they are introduced in their natural paths, or rather, that if they are introduced by other routes, an excessive amount of virus is required to produce a specific reaction. Varicella, a disease of the lymphatic system, does not enter the body by means of the blood stream and its virus has not acquired the property of invading the human organism by this path. It was this line of reasoning which led us to undertake this mode of therapy.

Camus[3] found, in his experiments on smallpox, that it required a very much larger amount of variola virus to produce this disease in rabbits when the intravenous, instead of the cutaneous, method was employed, and recently Noguchi[4] noted a similar phenomenon in experimenting with his testicular virus. The latter reports that in order to produce an eruption one thousand times more virus had to be introduced by way of the blood stream than when applied on the skin. It is interesting to note in this connection that Noguchi showed that the lymph glands harbored the virus; this may likewise hold true for varicella.

The vesicular lymph was given by us in other ways also, as will be seen by consulting the table. It will be noted that in a number of cases it was introduced intravenously after having been inactivated by exposure to a temperature of 56 C. for three-quarters of an hour. In sixteen instances the unheated lymph was injected intracutaneously; in ten, subcutaneously, and in a few cases was placed on the broken skin, on the mucous membrane of the nose, on the tonsils and buccal mucous membranes and on the mucous membranes of the nose and mouth. It seemed as if these methods might afford a simpler technic, and one which would avoid the somewhat difficult intravenous puncture.

About three years ago Herman[5] made an attempt to immunize infants under six months against measles by applying to the nostrils nasal mucus from children having the disease. He carried this out in forty cases and believed that he had brought about immunity in a certain number of instances. In the light of this report and in view of the simplicity of the method it seemed worth while to make a similar attempt on a small number of cases of varicella. In view of the fact that the vesicular lymph, which is known to contain the varicella virus, did not induce the slightest disorder when applied in this way, it was evident that no disturbance was to be apprehended from a similar use of nasal mucus. In three instances, therefore, the nasal secretion of varicella patients was applied to the nostrils; in three others the tonsillar secretion placed on the tonsils; and in six, the tonsillar and pharyngeal secretions were transferred to the nose, the pharynx and the tonsils. In none of these twelve cases was there any reaction whatever, either local or systemic. In two, secondary varicella developed in the course of the epidemic, from six to eight weeks later—one where the nasal secretion was employed and another where the tonsillar secretion was made use of. It is therefore evident that this method cannot be relied on to bring about protection. This is all the more striking in view of the fact that of these twelve cases only six came in intimate contact with varicella, so that we must consider that two of the six so treated failed to develop immunity to the disease.

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[3] Camus, L.: Compt. rend. Soc. de biol., 1913, 75, 344.
[4] Noguchi, H.: Jour. Exper. Med., 1918, 27, 425.
[5] Herman, C.: Arch. Pediat., 1915, 32, 503.

It will be seen from the table, however, that the number of these cases which came into intimate contact with the varicella were too few to allow us to judge of the success of these attempts at vaccination. Two facts, nevertheless, stand out in sharp relief: first, that no instance of primary varicella, even of the mildest type, resulted from these attempts at immunization and, second, that only the intravenous method was shown to afford adequate protection. The latter conclusion is inevitable in view of the fact that chickenpox developed about two months later in one of the three cases in which the lymph was placed on the broken skin (von Pirquet) and in two of the three cases in which it was applied to the nostrils. These few tests suffice to show the inefficacy of these simpler methods.

Incidentally, these tests furnish interesting information as to the portal of entry of the virus of varicella. The fact that the lymph from the vesicles, when applied to the broken skin or to the mucous membranes, led to no infection must cause us to consider this investigation from quite another standpoint. How are we to explain this lack of infectivity of the contents of the vesicles which are generally regarded as the source of infection and are used as the criterion in prescribing the duration of quarantine? There seems to be but one logical interpretation of these results, namely, that the lymph was in no instance applied to its natural portal of invasion. The only other possibility—that the virus is not present in the vesicle—is apparently refuted by our experience that immunity was brought about by injections of this fluid. What is then, Nature's path of invasion? These vaccinations afford strong testimony—of a negative character, it is true—for an answer to this question. In view of our results and the highly infectious nature of this disease, we are led to the tentative conclusion that the virus is air borne and that its portal of entry frequently is the respiratory tract.

SUMMARY OF PROTECTIVE IMMUNIZATION

Mode of Introduction of Lymph	Number of Cases*	Number Subsequently in Contact with Chickenpox	Number of Contacts Developing Chickenpox	Remarks
Intravenous	38	38	1	Chickenpox appeared in this case 41 days following prophylactic therapy
Intravenous (inactivated)	9	1	0	
Intracutaneous	16	5	1	No local reaction
Subcutaneous	10	0	—	
Skin (v. Pirquet)	3	3	1	Chickenpox 62 days after immunization
Nostrils	3	3	2	Chickenpox 44 and 62 days after immunization
Tonsils and mouth	3	3	0	
Tonsils, nose and mouth	4	0	—	

* There was no instance of signs or symptoms of varicella occasioned by the immunization.

Conclusions.

It is possible to bring about immunity to varicella by means of an intravenous injection of the contents of the vesicles. In thirty-eight instances in which this was carried out it failed to protect in only one case. Vaccinations of this kind induce neither local nor systemic reaction. The acquisition of immunity likewise indicates that the specific virus is contained in the vesicles. A simpler method of therapy, the application of the lymph to the broken skin or mucous membranes, failed to bring about satisfactory immunity. Like the first method it was followed by no unfavorable results.

These investigations have a secondary bearing as to the natural portal of entry of the varicella virus into the body. As we can exclude the skin and the mucous membranes in this connection, it would seem most probable that the virus enters by way of the respiratory tract and that contagion comes about through the air. This mode of infection would account for the almost unexampled communicability of this disease.

REPORT OF THE 1916 EPIDEMIC OF POLIOMYELITIS

From a Clinical and Laboratory Standpoint.*

JOSEPHINE B. NEAL, PHEBE L. DU BOIS, HARRY L. ABRAMSON AND ASSOCIATES.

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INTRODUCTION.

The meningitis division was established by Dr. Park in 1910 as a division of the Bureau of Preventive Medicine, to afford to the physicians of New York City expert assistance in the diagnosis and treatment of meningitis. In connection with this work, a great variety of meningeal conditions have been studied. Among these were about seventy cases of poliomyelitis before the epidemic; most of which were either nonparalytic or seen at least before the paralysis had developed. This had given us very good training in the correlation of the clinical picture and the spinal fluid findings necessary for the differential diagnosis of nonparalytic cases of poliomyelitis.

Early in June, 1916, it was noted that several cases of poliomyelitis occurred in Brooklyn, and that they were rather closely grouped. On June 9 a letter was written to the Bureau of Communicable Diseases calling attention to this fact.

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*A combination of three articles published in:
Arch. of Int. Med., Sept., 1917, 20, p. 341, and Sept., 1918, 22, p. 312.
Am. Jour. Med. Sc., Sept., 1916, 142, 3, p. 313.
Reprint Series, Dept. Health, City of New York, April, 1917, No. 55.

From this time on the number of cases increased rapidly and we were soon in the midst of the most extensive epidemic of poliomyelitis that has ever been studied. Not only was the number of cases the highest on record (8,978 cases being reported from June 1st to November 1st in New York City alone), but the mortality (26.9 per cent., New York) was higher than in any epidemic previously recorded. In such epidemics the mortality has not usually exceeded 15 per cent.

A very brief account of the history of poliomyelitis may be of interest.

RECORDED EPIDEMICS.

Although effort had been made for a long time to differentiate poliomyelitis and establish it as a clinical entity, the first important contribution to the study was a monograph published by Heine[1] in 1840.

Colmer,[2] in 1843, published an account of an epidemic of ten cases occurring in West Feliciana in 1841.

Strümpell,[3] in 1884, published an article differentiating the cerebral type of the disease for the first time.

Medin,[4] in 1890, published a study, which, with Heine's, ranks as a classic. Indeed, poliomyelitis is often called Meine-Medin's disease, from the epoch-making work done by them. Medin's work was based on epidemics in Sweden.

The first considerable epidemic in the United States occurred in Otter Creek Valley, Vt., in 1894, and was reported by Dr. Charles Caverly. About ten small epidemics were reported in the United States during the next twelve years.

In 1905 the first great epidemic occurred, over 2,000 cases being reported in Norway and Sweden. This was carefully studied by Wickman,[5] whose work ranks in importance with that of Heine and Medin. He arranged an excellent classification, developed the epidemiology and pathology, demonstrated the contagious character of the disease, and described, for the first time, the nonparalytic or abortive form.

In 1907,[7] a still larger epidemic, comprising about 3,000 cases, occurred in New York City.

In 1909, there was an outbreak in the Province of Santa Clara in Cuba.[8]

In 1909 and 1910, epidemics appeared in various parts of the United States, including the District of Columbia, Iowa, Massachusetts, Minnesota, Indiana, Pennsylvania, Kansas, Maryland, New Hampshire, New York, Rhode Island, Virginia, Washington and Wisconsin. These epidemics varied in size from 200 to 500 cases, sometimes even more.

During this time important experimental studies, referred to elsewhere, were made both in this country and abroad.

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1. Heine, Jacob: Boebachtungen über Lähmungszustände der untern Extremitäten und deren Behandlungen, Stuttgart, 1840; Spinale Kinderlähmung, Stuttgart, 1860.
2. Colmer: Am. Jour. Med. Sc., 1843, 5, 248.
3. Strümpell: Jahrb. f. Kinderh., 1884, 22, 173.
4. Medin: Verhandl. d. Xth internat. Med. Cong., 1890, 2, 6 Abt., p. 37.
5. Wickman, Ivan: Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907; Die akute Poliomyelitis, oder Heine-Medinsche Krankheit, Berlin, 1911.
6. Infantile Paralysis in Massachusetts During 1910. Monthly Bulletin Massachusetts State Board of Health, 1912.
7. Epidemic Poliomyelitis, Report, etc., on New York Epidemic, 1907, Nervous and Mental Disease, Monograph Series No. 6, New York, 1910.
8. Labredo and A. Recio: Sanidad y Beneficencia Boletin Oficial de la Secretaria Habana, March, 1910.

A study of infantile paralysis in Massachusetts during 1910,[6] reprinted from the *Monthly Bulletin* of the State Board of Health in 1912, includes clinical, epidemiologic and experimental work.

An excellent monograph by Peabody, Draper and Dochez,[9] in 1911, includes a careful clinical study and also considerable laboratory and research work.

Kling and Leviditi[10] published, in 1913, a very interesting epidemiologic study of the spread of the disease on two small islands, Djursö and Yxnö, near the coast of Sweden, where the movements of the people could be very closely followed. The progress of the disease either by direct contact or through carriers, was clearly shown.

F. E. Batten[11] made a study of the epidemiology of the disease in England and gives in the Lumleian Lectures for 1916 a careful summary of the most important work done along various lines.

PART I.

Clinical and Laboratory Work.

During the epidemic in New York in 1916, cases in which there was a disagreement between the department diagnosticians and the attending physicians were referred to us for diagnosis. This enabled us to study many atypical cases and to make some rather interesting differential diagnoces. From the middle of June until the middle of October we were called on in 461 cases in practically all of which poliomyelitis was suspected. Table 1 shows the final diagnoses with a comparison of the patients seen and the diagnoses for the corresponding time in 1915.

TABLE 1.

COMPARISON OF DIAGNOSES IN 1915 AND 1916.

	June 15 to Oct. 15, 1916	June 15 to Oct. 15, 1915
Anterior poliomyelitis	312	7
Anterior poliomyelitis, doubtful	29	..
Tuberculous meningitis	29	16
Epidemic cerebrospinal meningitis	18	11
Other purulent meningitides	8	5
Acute syphilitic meningitis	1	0
Meningism	18	8
Other diseases	46	18
Total	461	55

The increase shown in the number of meningeal conditions other than poliomyelitis indicates that such cases are incorrectly diagnosed at ordinary times when recourse is not had so freely to lumbar puncture.

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[9] Peabody, Draper and Dochez: A Clinical Study of Acute Poliomyelitis. Pub. Rockefeller Inst. for Med. Research, 1911.

[10] Kling and Leviditi: Etudes sur la poliomyelitis Aigié Epidemique, Pub. de l'Inst. Pasteur, Paris, 1913.

[11] Batten F. E.: Acute Poliomyelitis (Lumleian Lectures, 1916), Brain, June, 1916, p. 115.

Dissemination.—Animal experimentation has conclusively demonstrated that the virus of poliomyelitis is discharged in the secretions of the nose, throat and alimentary tract of patients suffering from the disease and in the secretions of the nose and throat of individuals in intimate contact with these patients.

In 1911 Kling, Petterson and Wernstedt first succeeded in inoculating monkeys with the virus obtained in washings from the nose, throat and alimentary tract of subjects who had died of poliomyelitis. Since then the experiment has been many times successfully repeated with washings obtained from living patients and also from their attendants.

Careful studies of epidemics in recent years have led to the conclusion that the mode of transmission of the disease is by actual personal contact, rather than through intermediary means, such as dust, flies and insects. The Stomoxys Calcitrans seems now to have been quite eliminated as a factor in the dissemination of poliomyelitis, though at one time much importance was attached to the part which this insect was thought to play in epidemics. It is conceivable that the common house fly may act as a passive carrier, as it does in typhoid fever; but its disease-bearing potentialities seem to be strictly limited to this passive role.

A careful study of recent epidemics also brings out the fact that while, not uncommonly, two or more children of one family may be attacked simultaneously by poliomyelitis, it much more frequently happens that but one case will occur in a household consisting of several children of susceptible age, all of whom may have been equally exposed to the contagion. A similar phenomenon is seen in epidemic meningitis. It is only to be explained on the theory that those who escape possess a natural immunity to the virus of poliomyelitis. The number of susceptible children must also be proportionately small. We do not understand either the mechanism of this immunity or the reason for it. However, the existence of such an immunity in the case of diphtheria infections has been clearly demonstrated by means of the Schick test and analogy gives rational grounds for the belief that it occurs also in polyiomyelitis.

Flexner has shown that the virus of this disease retains its virulence when subjected to high summer temperatures, to drying or to the action of weak chemicals which destroy ordinary bacteria. It posseses, therefore, considerable resistance, and this fact should be borne in mind with every case. Bright sunlight readily destroys the germ.

An attempt was made to follow up cases to determine how infectious poliomyelitis is, or how much natural immunity children have, by studying the number of secondary cases developing in families, a member of which was known to have the disease.

In following up 360 cases it was found that in 198 instances there were other children in the family who were, therefore, directly exposed to the disease. The children thus directly exposed numbered 443 and of these twenty-eight, or 6.3 per cent., developed the disease. This is in accordance with work done by Emerson[12] in Massachusetts and Hill in Minnesota, in 1909. The distribution by families is shown in Table 2.

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[12] Infantile Paralysis in Massachusetts in 1909. Bulletin Massachusetts State Board of Health, 1909.

TABLE 2.

OCCURRENCE IN CONTACTS IN SAME FAMILY.

Number of Children in Family	Number of Families	Children Developing Disease	Number of Children in Family	Number of Families	Children Developing Disease
2	72	13	6	9	1
3*	60*	9*	7	5	1
4	31	4	8	1	0
5	18	0			

* Among these families in two instances all three children developed the disease. These were the only instances in which more than two children in a family were affected.

Table 2 shows that a relatively large number of children must have a considerable natural immunity to poliomyelitis, since so many exposed closely to the disease escaped an attack.

Classification.—The classification of a disease so protean in its manifestations as poliomyelitis is unsatisfactory. To most of the laity, and to many physicians as well, poliomyelitis is known as a disease characterized by a frank, flaccid paralysis, its last stages being always mentally associated with the usual orthopedic brace. It may be well, therefore, to consider at this time those more atypical or abortive cases, in which paralysis is transitory or entirely absent because these so usually unrecognized, are the most potentially dangerous.

In his monograph on acute poliomyelitis, Wickman presents the following classification of its varieties which is self-explanatory.

1. Spinal poliomyelitic form.
2. Form resembling Landry's paralysis.
3. The bulbar or pontine form.
4. Encephalitic type.
5. Ataxic type.
6. Polyneuritic type.
7. Meningitic type.
8. Abortive type.

While this classification is quite comprehensive it is perhaps somewhat too complex for clinical use; a simpler classification is that of Peabody, Draper, and Dochez, which presents three groups, namely:

1. Abortive or non-paralytic types (cases presenting no paralysis at any time).
2. Cerebral group (embracing involvements of the upper motor neuron with resulting spastic paralysis).
3. Bulbar spinal group (presenting lesions of the lower motor neutron with flaccid paralysis).

A classification based on the anatomy and the degree of the lesion, is simple and quite easily used. We would suggest the following:

1. *Nonparalytic Type.*—Under this head are included cases in which the nerve cells are not sufficiently injured to produce paralysis. There may be weakness. Under this type should be classed meningitic cases and also those cases somewhat like tuberculous meningitis, but without motor disturbance which we have called encephalitic since the chief symptom seemed to be a depression of the sensorium. This is really only an accentuation of

the drowsiness and stupor charactertistic of the early stages. In these cases the motor cortical areas are not involved.

2. *Ataxic Type.*—The motor nerve cells are evidently not involved, but there is lack of coordination, ataxia, nystagmus, etc. In some cases an ataxic gait is the only sign of involvement of the central nervous system other than the sensory symptoms. The anatomic basis for this is proved by the postmortem findings of involvement of the cerebellum, Clark's column and the intervertebral ganglia. This type is very rare.

3. *Type with Cortical Paralysis.*—The upper motor neuron is affected, with resulting spastic paralysis. A true spastic paralysis is rare. More often are seen evidences of involvement of the upper motor neuron, increase of reflexes, or severe and prolonged convulsions. These convulsions are general and epileptic in character and may last for several hours.

4. *Type with Spinal or Subcortical Paralysis.*—The lower motor neuron is affected with resulting flaccid paralysis. This is, of course, the most common form and the first one recognized and described.

In comparing this classification with that of Wickham, Group 4 corresponds with Wickman's Type 1, and includes his Types 2 and 3, since Type 2 is always an involvement of the lower motor neuron and Type 3 usually is. Group 3 corresponds with Wickman's Type 4, when actual lesions of the upper motor neuron are present; Group 2 with Type 5, Group 1 with Type 8, including those cases of Types 6 and 7 in which there is no paralysis, while cases of Types 6 and 7, in which paralysis is present, would fall under Group 3 or 4, depending on whether the upper or lower motor neuron is involved.

This classification is based on our experience during the epidemic and cases were grouped under it after the epidemic was over. Had we been following it as the cases were studied, we could doubtless have made a more satisfactory grouping. Of course, cases sometimes fell in more than one group—Groups 2 and 4, most frequently.

The following is the classification of 512 cases:

Group 1	202	Mixed Groups:	
Group 2	4	Groups 2 and 4	3
Group 3	5	Groups 2 and 3	1
Group 4	296	Groups 3 and 4	1

Facial paralysis was rather common in this epidemic. Of 352 cases, there was facial paralysis alone in eighteen cases, combined with other paralysis in ten cases.

A manifestation of poliomyelitis difficult to classify is blindness.

Wickman[5] says: "I may appropriately here mention that the optic nerve also is in rare cases affected." Tedeschi found complete blindness with optic atrophy in the left eye of a chronic case and Wickman demonstrated optic neuritis in a recent case.

Three cases of blindness have been seen in poliomyelitis by the physicians of the meningitis division. In view of the rarity of the condition, a brief description of these cases may be of interest.

Case 1.—E. M., aged 3 years; illness began Sept. 23, 1911, with symptoms characteristic of poliomyelitis. Partial paralysis of the left arm and left side of face occurred with left internal strabismus. About 70 c.c. of clear cerebrospinal fluid were withdrawn October 14. The changes were characteristic of poliomyelitis: marked increase in cells, 98 per cent. mononuclears; bacteriology negative; albumin and globulin + +. October 24, the general condition was better but the child was blind. Early in November the sight began to improve and by the middle of December the child was perfectly well.

Case 2.—I. C., aged 4 years 10 months. Illness began July 1, 1916, with paralysis of legs and neck and ptosis of left eyelid. The paralysis of the legs was spastic in type. Blindness developed. The patient died Sept. 11, 1916.

Case 3.—R. J., aged 1 year. Illness began Aug. 31, 1916, with symptoms characteristic of poliomyelitis. There was no paralysis, but the child was blind (as nearly as could be determined) for three days. It made a complete recovery.

It is generally accepted that the most common cause of death is respiratory failure, due to the progressive paralysis of the muscles of respiration. The question of death occurring as a result of respiratory failure, or of cardiac failure due to the involvement of the respiratory or cardiac centers has received but little consideration. Wickman, referring to the occasional occurrence of Cheyne-Stokes respiration, says that it is undoubtedly due to the respiratory center being affected. He also mentions the possibility of the involvement of the vagus. Realizing that this question lies to a great extent in the realm of speculation, certain observations point to the desirability of giving it serious consideration. It is certainly possible to conceive from the pathologic findings that this condition may exist. . Cases occur in which the only lesions are in the bulb and pons, there being no involvement of the cervical regions of the cord where the nerves controlling the muscles of respiration take their origin.

During the epidemic, death occurred suddenly, without signs of cardiac failure, in a few cases in which there was no paralysis of the muscles of respiration. In one case death occurred without evidence of paralysis evidently from cardiac disturbance. For twenty-four hours preceding death there had been intervals of great irregularity of the heart, though the heart itself from the physical examination was apparently normal. Such a condition might conceivably arise from a progressive involvement of the vagus center in the medulla.

In several instances there was evidence of a transient involvement of the cardiac or respiratory centers, as shown by marked irregularity of the heart or respiration, which cleared up completely. As a specific example of a fatal case of this kind, the following may be cited:

I. F., aged 2 years. The illness began July 26, 1916, with facial paralysis. The spinal fluid showed pathologic changes, such as are commonly found in poliomyelitis. Death occurred July 31, no signs of paralysis of the respiratory muscles having developed. The microscopic pathology of this case showed the lesions of poliomyelitis present only in the pons and bulb.

The classification of this particular type is difficult. Of course the lesions occur in the pons and bulb. These cases especially when fatal are usually associated with paralysis of the cranial nerves—the facial or glossopharyngeal, abducens, etc. These are lower motor neuron lesions and are classified by us under Group 4. We are at a loss to know how to place a lesion of the vital centers.

The existence of this type of lesion does not seem to have been very clearly differentiated in the past. Even Wickman refers to paralysis of the respiratory muscles and lesions of the respiratory center without clearly distinguishing the conditions. Doubtless further study will throw more light on the clinical and pathologic side of this question.

It is maintained by some authorities that poliomyelitis is essentially a general infection and that symptoms of its effects on the central nervous system are entirely secondary in nature when not absent altogether. It has even been suggested that paralytic cases may be in reality the atypical ones. Those holding this view would in times of epidemics diagnose as poliomyelitis cases presenting merely respiratory or gastro-intestinal symptoms, particularly if a case of frank paralysis exists in the same family at the time. When we consider the prevalence of gastro-intestinal disturbances in children more especially during the summer season, such a conclusion is hardly tenable unless corroborated by characteristic findings in the spinal fluid.

Personal experience inclines us to believe that the changes in the spinal fluid commonly accepted as diagnostic of poliomyelitis do not exist independently of some definite clinical manifestation of involvement of the central nervous system. It does not seem possible that a disease accompanied by inflammatory reaction in the central nervous system and meninges of sufficient severity to produce changes in the spinal fluid could fail at the same time to afford some clinical evidences of nerve involvement. Therefore, in the past we have made it a rule to consider the possibility of poliomyelitis and to perform lumbar puncture only in those cases presenting symptoms referable to the nervous system, such as hyperesthesia, altered reflexes, stiffness of the neck, Kernig's or the spinal sign, Macewen's sign, etc. When none of these signs was present we have usually considered a lumbar puncture unjustifiable. In the few cases in which we have performed it, the fluids have been normal. In the presence of a normal spinal fluid and in the absence of these signs we have always definitely excluded poliomyelitis.

During the recent epidemic we performed punctures irrespective of our own opinion as to the diagnosis to clear up doubtful cases and the results have justified our earlier views.

At the other extreme from those regarding poliomyelitis as a general infection and the paralytic cases as atypical, are physicians few in number to be sure who regard as suffering from poliomyelitis only those patients with a frank paralysis. The following reasons seem to prove quite conclusively the existence of the nonparalytic type:

1. The filtered washings of the nose and throat of such patients in a considerable number of instances have produced typical poliomyelitis in monkeys.
2. The findings in the spinal fluid in those cases are identical with the findings in poliomyelitis with paralysis.
3. The findings in the spinal fluids in cases of meningism in gastroenteritis, pneumonia, the exanthems, etc., are usually quite distinctly different.
4. The serum from nonparalytic patients possesses the same neutralizing power as the serum from paralyzed patients.

Incubation Period.—The incubation period of poliomyelitis has not been definitely determined. It is considered by Wickman to be from one to four days and by Müller to be from five to ten days, averaging about a week. This corresponds to the incubation period of the disease as experimentally produced. Some observers, as Peabody, Draper, and Dochez, describe a true prodromal stage which they define as the period before the appearance of paralysis. As it is well known that many cases of poliomyelitis remain entirely free from all paralysis, it would seem unwise to date the incidence of the disease, even in the paralytic form, from the appearance of paralysis. We are, therefore, not justified in speaking of a true prodromal stage.

Symptoms of Onset.—The initial symptoms are much the same in all types of the disease, and they may be quite as severe in the nonparalytic as in the paralytic forms. There is a type of onset described by Kling, Leviditi and others, which we have sometimes observed. In this form the disease is ushered in by somewhat indefinite symptoms of an intestinal or anginal nature. A remission of from one to several days then occurs, to be followed by a return of all symptoms, and usually by an accompanying paralysis.

While the onset may rarely be insidious, in the vast majority of cases it is very abrupt. Fever is probably the most constant as well as usually the first symptom. As a rule, it is high and of comparatively short duration falling by crisis or by lysis. Next to fever, the most frequent symptom is a pronounced hyperesthesia or diffuse tenderness over the whole body. This is, perhaps, most marked in the legs and along the spine. There is also a decided drowsiness and the patient manifests great irritability when disturbed. Headache and vomiting are common. Constipation or diarrhea may be present; in our own experience, the former being more frequent. Retention of urine sometimes occurs and this possibility should be constantly borne in mind. Convulsions, twitchings of groups of muscles, delirium and pains in the neck, back, joints and limbs are other symptoms which may appear. Meningeal symptoms are pronounced in a fairly large portion of cases. These are, anteroposterior stiffness of the neck; Kernig's sign or the spinal sign of Peabody, Draper and Dochez; Macewen's sign, or change in the percussion note over the lateral ventricles, due to distention with fluid. The reflexes may be exaggerated, but usually they are diminished or abolished. They are frequently unequal. The pupillary reflex is, as a rule, retained. Following these symptoms, paralysis may develop, appearing most commonly about the second day though it may be delayed as late as the second week.

While occasional references to herpes zoster associated with polilomyelitis are noted in the literature, it would seem that the association must be accidental, or else especially characteristic of certain epidemics. In this epidemic no instances were seen by us of this manifestation, nor is there any record, to our knowledge, of herpes zoster occurring in experimental poliomyelitis. If it were due to the virus of poliomyelitis, it would seem that this manifestation would occur occasionally, at least, in monkeys artificially infected, inasmuch as in these cases the posterior root ganglia invariably show the typical pathologic lesions.

Differential Clinical Diagnosis.—In the first twenty-four to forty-eight hours after its onset, polilomyelitis must be differentiated from the early stages of several diseases presenting a similar clinical picture, especially epidemic meningitis, or mild purulent meningitis, and also from a meningism accompanying pneumonia or other infection.

When seen a week or more after onset, cases of poliomyelitis especially if presenting cerebral symptoms, must be distinguished from tuberculous meningitis.

Between *purulent meningitis* in an early stage and early cases of poliomyelitis the differential diagnosis is fairly easy in typical cases, but in the many atypical cases the following points are significant:

1. The history of a recent illness prior to the present attack. In this case a history of a gastro-enteritis or an anginal attack three or four days before the onset is much more suggestive of poliomyelitis than of meningitis. On the other hand a history of an otitis media, an operation on the nose or throat, or a severe injury to the head with possible fracture of the skull is suggestive of meningitis due probably to the pneumococcus or streptococcus.

2. The temperature in poliomyelitis is usually higher at the beginning but falls more quickly than in meningitis.

3. There is usually greater hyperaesthesia in poliomyelitis. The reflexes are more likely to be unequal in poliomyelitis, but the pupillary reflexes are very seldom lost.

4. In meningitis there is usually greater stiffness of the neck, and a more pronounced Kernig sign. Delirium is far more common than in poliomyelitis. A hemorrhagic eruption or herpes, if present, strongly suggests meningitis.

In differentiating between *tuberculous meningitis* and poliomyelitis, one should remember that while the onset is usually sudden in poliomyelitis and gradual in tuberculous meningitis, yet some few cases of poliomyelitis give a history of a gradual onset and occasionally tuberculous meningitis begins abruptly.

In the cases of poliomyelitis resembling tuberculous meningitis the stupor is usually not so profound, there is no projectile vomiting, the pulse is usually regular. The greatest mortality in poliomyelitis is in the early stages of the disease so that the progress of the case in a patient who outlives the first week or ten days is generally toward recovery.

A paralysis of the eye muscles often develops in tuberculous meningitis. Other paralyes may also develop, as paralysis of the face or arms, but these symptoms are usually transitory in tuberculous meningitis.

Between *meningism* and poliomyelitis the differential diagnosis is much more difficult until the underlying cause of the meningism develops. Even then it may be a question whether the case is one of meningism caused by a primary pneumonia, gastro-enteritis, or some other disease, or whether the supposed primary infection may not itself be a complication of a poliomyelitis.

In all cases where the clinical examination does not furnish indisputable evidence of the nature of the disease the examination of the spinal fluid will be of the most valuable assistance in making the diagnosis.

Outcome of Cases—

While about 2,000 poliomyelitis fluids were examined, only 500 cases were carefully studied. Five hundred is accepted by statisticians as a number furnishing absolutely reliable data from which to draw conclusions. It seemed better, therefore, to take this number and make careful studies, combining clinical data with the spinal fluid findings, rather than to try to use a larger number and study the data less thoroughly.

The results at the end of two months in 507 cases studied were as follows:

Class 1 (Nonparalytic): Of 202 cases, 173 recovered completely; 29 had weakness remaining.

Class 2 (Ataxic): Of 4 cases, 4 patients recovered.

Class 3 (Cortical or upper motor neuron paralysis): Of 5 cases, 5 patients died.

Class 4 (Spinal or lower motor neuron paralysis): Of 296 cases, 32 recovered completely; 32 recovered with weakness; 130 had paralysis remaining after two months; 102 died.

The cases under Classes 2 and 3, of course, are too few to make it possible to draw conclusions.

Of Class 4, the spinal or lower neuron type, 130, or 67 per cent. of the 194 surviving, showed a residual paralysis at the end of two months.

It will be noticed that of these 507 cases, 202, or 40 per cent., were nonparalytic.

The relatively large proportion of such cases in our report is due to the fact that this type of case was the one giving rise most frequently to differences of opinion, and at these times we were called upon to help in making the diagnosis.

TREATMENT.—Thus far no specific treatment for poliomyelitis has been successfully developed. In the minds of the general public and of most physicians there exists a close analogy between the use of serum in epidemic meningitis and in poliomyelitis. The success of this treatment in the former disease has given rise to hopes for the success of the same method in poliomyelitis. The pathology of the two conditions is, however, quite different. In acute purulent meningitis the process is limited almost entirely to the meninges, the brain and cord substance being little if at all involved, though there may be some secondary congestion. In poliomyelitis, however, the pathologic picture is reversed. Here, the brain and cord substance is mainly involved, whereas the inflammation in the meninges is secondary. Injection of a foreign substance into the slightly inflamed meninges sets up in most cases an acute aseptic meningitis, as is shown by changes in the spinal fluid and clinically by the increased temperature, rigidity of the neck and other signs of meningeal irritation. It is possible that this increased inflammatory reaction may tend to accentuate indirectly the inflammatory changes already existing in the subjacent substance of the brain and cord. That this reaction may be harmful is borne out by the results of animal experimentation, which are described in another place. On the other hand, certain workers feel that a beneficial phagocytosis may result from the increased leukocytes. In meningitis the injection of serum into actively inflamed meninges is not followed by an increase in the inflammatory reaction as is evidenced by the clearing up of the fluid and the amelioration of the clinical symptoms in favorable cases.

As already stated, it seems to us that the setting up of an acute aseptic meningitis may be harmful. As to the specific neutralizing power of the serum this would have to be exerted very speedily, since the damage by the disease occurs so often within forty-eight to seventy-two hours after the onset of symptoms. Following the injection of antimeningitis serum, tetanus antitoxin and other antiserums such a speedy curative effect is not observed.

The unanimous opinion of the physicians of the meningitis division is voiced in the preceding discussion.

The members of the meningitis division did not administer serum in a very large number of poliomyelitis cases, because the early experiences were so discouraging that they did not feel that they could urge its administration. The reaction following the introduction of the serum in the cases of the patients who survived was often so severe that it was thought the inoculation might have been an injurious factor in some instances.

They feel that the best results in early cases are obtained by complete rest, with lumbar puncture for the relief of signs of meningeal irritation. Symptomatic and hygienic treatment should, of course, be carried out. The importance of rest cannot be too strongly emphasized. That form of treatment would need to be of definite and well-established value that could justify the frequent disturbance of a desperately sick child for the administration of treatment intraspinally.

The results obtained during the epidemic of 1916 do not confirm the hope that the intraspinal injection of serum from cured patients would prove to be of value in the treatment of poliomyelitis.

EXAMINATION OF SPINAL FLUID—

In 1908, Dr. Martha Wollstein[13] published a biologic study of the cerebrospinal fluid in anterior poliomyelitis. This included bacteriologic and complement fixation work, rather than cytologic and chemical studies, and refers in detail to still earlier work along those lines.

Gay and Lucas,[14] in 1910, published one of the earliest and most important studies, embracing cytologic and chemical work on the spinal fluid, both from human cases and from experimental disease in monkeys.

The spinal fluid was carefully studied in the monograph published by Peabody, Draper and Dochez,[9] already referred to, and this work was continued by Fraser.[15]

The fluids examined at the Research Laboratory during the 1916 epidemic included those withdrawn by the members of the meningitis division, those sent in by private physicians and those withdrawn at the Willard Parker Hospital, and at the Kingston Avenue Hospital. To take care of the fluids at the latter place, it was necessary to establish a special laboratory there.

MACROSCOPIC APPEARANCE.—The spinal fluid in poliomyelitis is usually increased in amount, and escapes under pressure. It is clear or slightly hazy in appearance, and sometimes shows the fibrin web formation, which was formerly considered pathognomonic of tuberculous meningitis. The "ground glass" appearance seen in the fluid when viewed with transmitted light is also found in the fluids of tuberculous meningitis, in early purulent meningitis with a slight cellular reaction, and in normal fluids where there is a small amount of blood present, and is therefore of little more significance than the increased quantity.

Two rare types of spinal fluids sometimes occur in poliomyelitis when the hemorrhagic process has been more than usually severe. The first of these types is of the true hemorrhagic character, the red blood cells being evenly distributed throughout the field. When collected in successive tubes the specimens of this type are all homogeneous, showing no change in the intensity of the hemorrhage. This serves to differentiate such specimens from bloody fluids obtained by the accidental puncture of a vein. The second of these rarer types of poliomyelitis spinal fluids shows evidence of an older hemorrhage by a characteristic yellow color and spontaneous coagulation, which illustrate the so-called syndrome of Froin. Both these types of fluid occur in other conditions and are therefore not pathognomonic of poliomyelitis.

TECHNIQUE OF EXAMINATION.—The technic which we employ in the further examination of spinal fluids is as follows: All clear or slightly cloudy fluids are centrifuged for an hour at high speed. From the sediment spreads are made, taking care to use, as nearly as possible, the same area on the different slides. The sediments of clear fluids are stained by the Ziehl-Nielson method for the tubercle bacillus; the sediments of slightly cloudy fluids are stained both for the tubercle bacillus and by the Gram method. Smears are also made for special blood stains in order to study the cells. (See below.)

The chemical tests that we use are the nitric acid ring test for albumin and the Noguchi butyric acid test for globulin. The albumin and the globulin practically always run parallel, but they serve as a check on each other. The small amount of albumin and globulin present in normal fluids is marked ±. The signs +, +|, ++, +++, ++++ represent increasing amounts and serve as a rough quantitative estimation. The presence of glucose is tested by using an equal amount of Fehling's solution and spinal fluid, and it is marked with regard to the speed and the amount of reduction as —, ±, +, ++, +++. The globulin reaction and the reduction of Fehlings should not be read for at least half an hour.

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[13] Wollstein: Jour. Exper. Med., 1908, 10, 476.

[14] Gay and Lucas: The Archives Int. Med., 1910, 6, 330.

[15] Fraser, F. R.: A Study of the Cerebrospinal Fluid in Acute Poliomyelitis. Jour. Exper. Med., 1913, 18, 242.

Taking up these points somewhat in detail, let us first consider the cytology. The increase in cells varies very greatly both in different cases and in the stage at which the puncture is done. From the stained sediment, we can estimate the increase in cells as slight, moderate, great, or very great. We do not feel that the exact number of cells has more than academic interest. The cell count in poliomyelitis and tuberculous meningitis, while on the average higher in the latter, overlaps for so wide a range as to render the differential diagnosis by this point most unreliable. Moreover, while some claims to the contrary have been made, it is not our opinion that the number of cells in poliomyelitis fluids bear any relation to the prognosis of the case. We have made a fair number of cell counts in connection with the work and we find that, on the whole the results obtained by a general estimate check up fairly well with those given by cell counts. Our counts have varied from slightly above normal, 15-20, to 1,200. The counts tend to fall off after the first week and by the end of the second week have fallen to practically normal in nearly all cases.

From the stained sediments we also estimate the percentage of mononuclears and note the presence or absence of endotheliod and polyform cells. The cells usually show a preponderance of mononuclears, but in a few instances there are over 50 per cent. of polymorphonuclears. It has been stated that early in the disease there is an excess of polymorphonuclears, which later is replaced by mononuclears. A large number of early fluids on the second, third and fourth days were examined by us. Out of a series of 2,000 fluids, many of them early, the polymorphonuclears predominated in only 39 cases, as follows: In four cases on the second day, in six on the third day, in one on the fourth day, in five on the fifth day, in three on the sixth day, in seven on the seventh day, and so on as late as the twenty-first and twenty-seventh days. We think, therefore, that an excess of polymorphonuclears represents a special type of reaction—and is not characteristic of a stage of the disease. There are large mononuclear cells that seem to be endotheliod in type that seem to us to occur more frequently in poliomyelitis than in other conditions. The so-called polyform cells are also found. Often, even in fresh fluids, the cells are so degenerated that it is difficult to classify them.

The stained smears are also searched for the presence or absence of bacteria. Cultures are made from all specimens. In the case of uncontaminated spinal fluids the cultures have been uniformly negative.

The chemical tests show that the albumin and globulin are usually slightly to moderately increased. In 141 fluids, 99 were marked + or +|; 36, ++ or ++|; 5, +++; and 1, ++++. The increase in albumin and globulin persists after the cell count has returned to normal. Fehling's, as has been stated before, is practically always well reduced. The fluids that show a poorer reduction are usually those with the larger amount of albumin and globulin.

Some quantitative work has been done on the chemistry of the poliomyelitis fluids, using the micro-chemical methods, which are described in

Part II. In brief, I may state that it has been found that the *total nitrogen* runs 20-30 mgms. per 100 c. c. Of this total nitrogen about one-half is non-protein and about one-fourth is urea.

Creatin—0.5 mgm. in 100 c.c. fluid.

Creatin—0.3 mgm. in 100 c. c. fluid.

Sugar is slightly lower than in the blood—.05 per cent. to .08 per cent. All poliomyelitic fluids show traces of cholesterol.

Laboratory Differential Diagnosis.—In the differential diagnosis of poliomyelitis fluids the slightly cloudy fluids must be differentiated from those of early cases of purulent meningitis and from the slightly cloudy fluid that occasionally occurs in tuberculous meningitis; the clear, or practically clear, fluids from rare early cases of a purulent meningitis, tuberculous meningitis, syphilis of the central nervous system, especially acute syphilitic meningitis, and meningism. Other rare conditions might be mentioned, but these are the most important.

In early cases of *purulent meningitis* the spinal fluid shows a varying degree of cloudiness, except in very rare instances, when it may be clear. A greater increase in albumin and globulin is usually found here than occurs in poliomyelitis, with a poorer reduction of Fehling's solution. The cells in these fluids of purulent meningitis are 90 per cent. or more polymorphonuclears, and the etiological organism is always found except in the mildest cases. In certain mild cases of meningitis—probably of the epidemic variety—the meningococci may never be positively demonstrated in the fluid. In purulent meningitis, due to other organisms, these organisms practically always appear later. In one instance only we have seen a clear fluid from an early case of epidemic meningitis of about eighteen hours' standing. Although the cellular reaction was so slight, the meningococcus was demonstrated to be present in the fluid by smear and culture.

The fluid in *tuberculous meningitis* most nearly resembles that of poliomyelitis. It is practically always clear with a cellular increase consisting largely of mononuclears, though in very acute cases it may be distinctly cloudy with an excess of polymorphonuclears. Fortunately, in these cases, the tubercle bacillus is usually easy to demonstrate. The number of cells per cubic millimeter is usually greater than in poliomyelitis; the increase in albumin and globulin is more marked, and the reduction of Fehling's is not so good. In rare instances, when clinical signs are confusing and when the results of the cellular examination and chemical analysis are indefinite, and it is impossible to demonstrate the tubercle bacillus in the fluid, a positive diagnosis must wait upon the results of animal inoculation.

The fluid of an acute *syphilitic meningitis* closely resembles the fluid of a poliomyelitis, and the clinical signs are also confusing. The Wassermann reaction is the best method of differentiating the two conditions. Of course, a positive Wassermann would not rule out a poliomyelitis in an old syphilitic condition, but this, combined with the clinical condition and the progress of the case, makes one reasonably sure of the diagnosis. It was suggested at first that the products of degeneration present in the spinal fluid of poliomyelitis cases might give a non-specific Wassermann reaction. Tests in about 350 cases, made by Miss M. Wilson, have demonstrated that this does not occur.

The fluid of *meningism* is clear, increased in amount and practically always normal in character. The few exceptions to this that we have found in a large series of cases have fallen mainly into three groups: fluids from cases with prolonged and severe convulsions; fluids in severe whooping cough; and fluids removed just prior to death. In these cases there has sometimes occurred an increase in cells, or in albumin and globulin or in both. In convulsions, there is probably oedema, in whooping cough there are probably minute hemorrhages, and just before death there may be circulatory changes to account for this increase.

Among additional tests Lange's colloidal gold test has been used to some extent by workers to whom we have given specimens of fluid, and it would seem that it may be helpful in differentiating the fluids of poliomyelitis from those of tuberculous meningitis and from such fluids of meningism as are not normal in character.

The following chart and tables are self-explanatory:

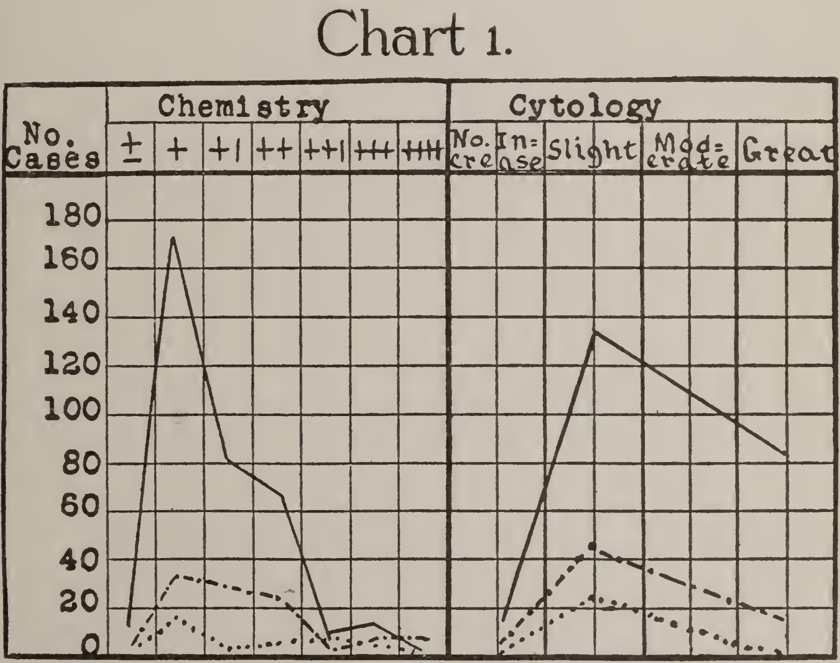

CHART 1.—Showing the variations in the chemistry and cytology of the cerebrospinal fluid in poliomyelitis. Upper curve ————————, first week; middle curve —.—.—.—.—.—., second week; lower curve ...., subsequent weeks. An idea may be gained from these curves as to the significance of the laboratory findings in the first and second and subsequent weeks. In the cytology the curves are nearly parallel, showing no tendency for greater cytology at any one time than another. There were relatively more cases with low chemical findings in the first week than in the later periods. but low chemical findings predominated in all three periods.

TABLE 3

RELATION OF DAY OF PUNCTURE TO LABORATORY FINDINGS

Day of Puncture.	Cytology					Chemistry						
	No. of Fluids.	No Increase.	Slight	Moderate.	Great	±	+	+|	++	++|	+++	++++
1	2	...	1	...	1	..	1	1	..	..	..	....
2	52	2	22	17	11	2	30	13	3	2	1	1
3	63	2	20	21	20	3	29	14	13	1	2	1
4	71	4	29	22	16	2	37	17	9	1	5	....
5	77	3	24	30	20	2	34	14	20	4	2	1
6	50	3	25	11	11	1	28	11	8	..	2	....
7	39	2	16	13	8	1	16	12	7	1	2	....
8	27	...	12	8	7	1	10	5	6	1	4	....
9	20	...	8	8	4	1	12	4	2	1	..	....
10	15	2	7	6	..	1	3	4	4	..	..	3
11	9	1	3	2	3	..	1	5	2	..	1	....
12	15	...	10	3	2	1	5	3	1	1	3	1
13	11	1	4	4	2	1	3	1	3	1	2	....
14	6	1	3	1	1	..	..	2	2	2	..	....
15	5	...	3	2	..	..	1	1	3	..	..	....
16	2	...	2	...	..	..	..	..	1	..	..	1
17	5	...	5	...	..	..	4	..	1	..	..	....
18	1	...	...	1	..	..	..	..	..	1	..	....
19	2	...	1	1	..	..	1	..	1	..	..	....
20	2	...	2	...	..	..	1	..	..	1	..	....
21	6	...	2	3	1	..	2	1	2	1	..	....
23	1	...	...	1	..	..	..	1	..	..	..	....
24	1	...	1	...	..	1	..	..	..	..	..	....
25	1	...	1	...	..	..	..	1	..	..	..	....
27	1	...	1	...	..	..	1	..	..	..	..	....
28	1	...	1	...	..	..	..	1	..	..	..	....
30	4	...	2	1	1	..	1	2	..	1	..	....
35	3	2	1	...	..	..	1	..	1	1	..	....
40	2	...	1	1	..	..	2	..	..	..	..	....
41	1	...	1	...	..	..	..	..	..	1	..	....
43	1	...	1	...	..	..	1	..	..	..	..	....
48	1	...	...	1	..	..	1	..	..	..	..	....
50	3	...	2	1	..	..	2	1	..	..	..	....
	500	23	211	158	108	17	227	114	89	21	24	8

The great majority of cases show a slight or moderate increase in the cells and a + or +| chemistry. The pathologic changes in the fluid both as to cytology and chemistry persist longer than has commonly been believed.

TABLE 4

RELATION OF CYTOLOGY TO CHEMICAL FINDINGS

CYTOLOGY		ALBUMIN AND GLOBULIN						
		±	+	+|	++	++|	+++	++++
No increase	23	1	12	7	3	0	0	0
Slight	211	9	105	39	35	9	11	3
Moderate	158	5	73	35	31	5	6	3
Great	108	2	37	33	20	7	7	2
	500	17	227	114	89	21	24	8

The chemistry does not run parallel with the cytology, as is clearly shown in the table.

TABLE 5

RELATION OF CONDITION AT THE TIME OF PUNCTURE TO FINDINGS

		CYTOLOGY				CHEMISTRY						
		No Increase	Slight	Moderate	Great	±	+	+|	++	++|	+++	++++
No paralysis	41	5	15	15	6	2	26	6	3	1	1	2
With weakness	261	11	102	83	65	12	121	54	50	13	8	3
With paralysis	198	7	94	60	37	3	80	54	36	7	15	3
	500	23	211	158	108	17	227	114	89	21	24	8

A majority of cases had slight increase in cells. A majority of cases had one plus chemistry. There is no relation between the paralytic condition and the spinal fluid findings, either as regards cytology or chemistry.

TABLE 6

RELATION OF CELL INCREASE TO OUTCOME; CONDITION AFTER APPROXIMATELY EIGHT WEEKS.

		Cytology		Complete Recovery.	Weakness or Recovering Paralysis.	Paralyzed.	Dead.	Mortality Per Cent.
Lumbar puncture in first week of illness.........	354	No increase....	16	9	0	4	3	19
		Slight.........	137	57	20	34	26	19
		Moderate......	114	50	10	25	29	25
		Great.........	87	35	11	23	18	20
Second week.....	103	No increase....	5	0	1	2	2	40
		Slight.........	47	18	9	15	5	10
		Moderate......	32	15	2	4	11	34
		Great.........	19	7	4	5	3	16
After second week	43	No increase....	2	1	1	0	0	0
		Slight.........	27	10	1	12	4	15
		Moderate......	12	3	2	6	1	8
		Great.........	2	2	0	0	0	0
	500		500	207	61	130	102	....

In the first and second weeks the cases with a moderate increase (from about seventy-five cells to 150 cells) had a higher mortality than the cases with a slight increase, or the cases with a great increase. It would be safe to conclude from this that a high cell count does not carry with it a bad prognosis.

TABLE 7

RELATION OF CHEMISTRY TO OUTCOME

		Chemistry.	Complete Recovery.	Weakness.	Paralyzed.	Died.	Mortality, Per Cent.
First week	354	±	6	1	1	3	27
		+	91	18	37	29	16
		+|	27	14	21	20	24
		++	19	6	20	15	25
		++|	1	1	4	3	33
		+++	2	1	6	5	35
		++++	1	1	0	1	33
			147	42	89	76	
Second week	103	±	4	0	0	1	20
		+	13	5	7	9	26
		+|	6	4	8	6	25
		++	9	4	5	2	10
		++|	3	0	3	0	0
		+++	3	1	3	3	30
		++++	2	0	0	2	50
			40	14	26	23	
Third week	43	±	0	0	0	1	100
		+	9	1	7	1	5½
		+|	2	2	4	0	0
		++	6	2	1	0	0
		++|	3	0	2	1	16
		+++	0	0	0	0	0
		++++	0	0	1	0	0
			20	5	15	3	
	500		207	61	130	102	

Of course with the smaller numbers, the mortality percentage means practically nothing. In the first week, cases with a + chemistry did better than those with a higher chemistry, but this did not hold in the second week. It would seem safe to conclude from this that the chemistry has no very definite prognostic value.

TABLE 8.

RELATION OF CONDITION AT TIME OF PUNCTURE TO OUTCOME; CONDITION AFTER APPROXIMATELY EIGHT WEEKS.

Lumbar Puncture in		Condition at Time of Puncture		Complete Recovery.	Weakness or Recovering Paralysis.	Paralysis.	Dead.
First week of illness.......	354	No signs of paralysis....	31	25	2	1	3
		Weakness or other marked symptoms...	197	108	25	30	34
		Paralyzed in some part...	126	18	14	55	39
Second week...	103	No signs......	8	6	1	1	0
		Weakness.....	48	28	8	7	5
		Paralyzed.....	47	6	7	18	16
After second week........	43	No signs......	2	2	0	0	0
		Weakness.....	16	11	1	1	3
		Paralyzed.....	25	3	3	17	2
	500		500	207	61	130	102

This table shows that while, after the first week, as is obvious, the chances of recovery are better, nevertheless, even later, it is quite impossible to make a prognosis that is at all definite.

TABLE 9.—SUMMARY OF 500 CASES.

TYPES OF CASES.		CYTOLOGY				CHEMISTRY							OUTCOME			
		No Increase.	Slight.	Moderate.	Great	±	+	+|	++	++|	+++	++++	Complete Recovery.	Weak.	Paralyzed.	Died.
Class 1 No paralysis........	202	12	77	65	46	12	103	36	35	8	5	3	173	29	...	...
Class 4 Flaccid paralysis....	296	11	133	92	62	5	123	77	54	13	19	5	32	32	130	102
Class 2 Ataxic............	2	....	1	1	....	....	1	1	....	....	....	....	2	....	....	....
	500	23	211	158	108	17	227	114	89	21	24	8	207	61	130	102

The cases were about evenly divided between two types—paralytic and nonparalytic.

Both types in the majority of cases had slight increase and one plus chemistry.

The paralyzed type in the majority of cases remained paralyzed.

The nonparalyzed type in the majority of cases recovered.

Out of 500 cases, in most there was a slight to moderate increase; in most there was one and one and one-half chemistry; in most there was recovery.

The percentage of those that made complete recovery after being paralyzed is 12.2.

The complete recovery among those not paralyzed is 76.2 per cent.

PART II.

RESEARCH WORK.

1. Gold Chlorid Test.

J. B. NEAL AND H. JONES.

Zsigmondy,[16] following an exhaustive study of the subject of the "coagulating" action of electrolytes on metallic colloidal solutions, was able to find a definite measure of the protective action of certain colloids, especially proteins, on the precipitation of gold suspensions by sodium chlorid. The degree of protection was specific for each protein he examined.

By using this general method he was unable to distinguish between syphilitic and normal serums.

Lange[17] proceeded further and found that normal spinal fluids, suitably diluted with a 0.4 per cent. solution of sodium chlorid, cause no alkalination in suitable solutions of colloidal gold, and abnormal spinal fluids cause partial or complete precipitation of colloidal gold, with resultant color changes occurring in curves, which tend to be almost specific for certain diseases, particularly those of syphilitic origin.

This specificity is characterized by maximal color changes within dilution zones.

Fluids from different types of meningitis give reactions with greater intensity in higher dilutions.

Paretic fluids cause complete flocculation in the first four to six dilutions.

Tabes and cerebrospinal lues give maximal reactions in fourth to fifth dilutions.

We have Lange results on 114 poliomyelitis fluids (Table 10).

Colloidal Gold Test.—Into the first of eleven test tubes put 0.9 c.c. of fresh, sterile 0.4 per cent. sodium chlorid solution. Into each of the remaining tubes put 0.5 c.c. of the 0.4 per cent. sodium chlorid solution. Add to the first tube 0.1 c.c. of the spinal fluid to be tested. Mix well.

Transfer 0.5 c.c. of the resultant 1 to 10 dilution of spinal fluid to the second tube Mix well.

Transfer 0.5 c.c. of the resultant 1 to 20 dilution of spinal fluid to the third tube. Mix well.

Proceed in this manner up to and including the eleventh tube.

By this method a series of dilutions of spinal fluid is secured, in geometrical progression, ranging from 1 to 10, to 1 to 5,120.

Now add to each tube 2.5 c.c. of colloidal gold solution.

Shake each tube thoroughly and do not read for twelve hours.

The various types of color changes seen in a positive gold reaction are indicated by numerals as follows.

Complete decolorization	5	Lilac or purple	2
Pale blue	4	Red-blue	1
Blue	3	Brilliant red-orange	0

A normal fluid would remain brilliant red-orange color and would, therefore, read 00000000000 or 1110000000.

A poliomyelitis fluid would remain brilliant red-orange color in the first two tubes, slightly bluish in the third, purple in the fourth tube, and again bluish in the fifth, returning to normal red-orange in the sixth tube, and would, therefore, read 00123000000—being a weak syphilitic curve.

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[16] Ztschr. f. Anal Chem., 1901, XL, 697.

[17] Berl. klin. Wchnschr, 1912, XLIX, 897; Ztschr. f. Chemotherap., 1913, I, 44.

A meningitis fluid unchanged in the first two tubes and ranging from this to colorless in the ninth tube and back to original in the eleventh tube, would read 00011245210.

Charts showing these typical reactions and readings are appended.[18]

Chart 2 shows the three curves of paretic, meningitic and syphilitic spinal fluids, according to the Bulletin of the Johns Hopkins Hospital, referred to.

Charts 3 to 8 show various poliomyelitis curves of fluids of our own.

Chart 9 is the composite of 3 to 8, giving the average curve for 114 fluids.

Our own fluids fell into six different groups, depending roughly on the albumin and globulin content. These are shown in Charts 3 to 8, while Chart 9 is a composite.

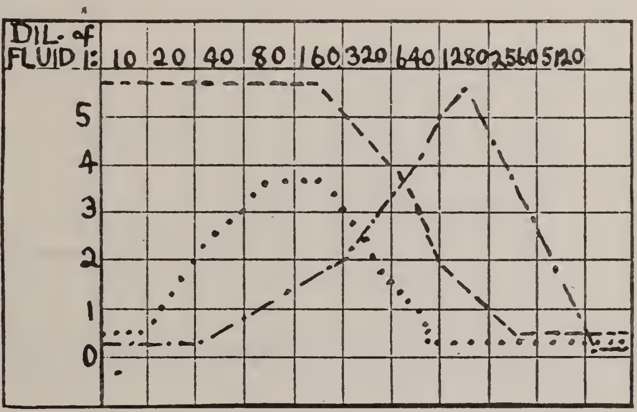

Chart 2.—Curves of cases of paretic, meningitic and syphilitic spinal fluids, reported in Bulletin Johns Hopkins Hospital, 1915, 26, 298. Dilution of fluids in each instance as shown in figures at the top of the chart, 1 : 10, etc. Paretic, 5555531000; syphilitic, 0123321000; meningitic, 00011245210.

Explanation of lines : – – – – – paretic ; . . . . . syphilitic ; — · — · — · meningitic.

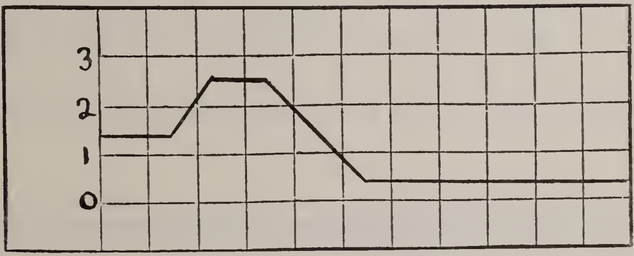

Chart 3.—Curve in forty of our poliomyelitic fluids: 1122100000.

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[18] These charts are taken from Bull. 298, Johns Hopkins Hosp., 1915, 26.

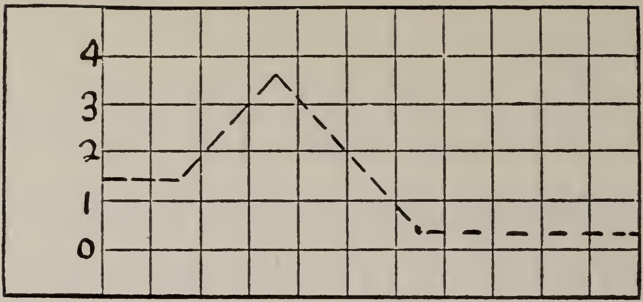

Chart 4.—Curve in eight of our poliomyelitic fluids: 1123210000.

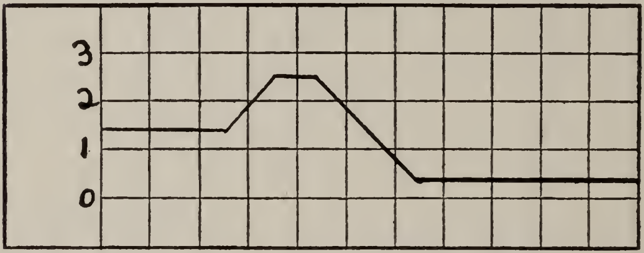

Chart 5.—Curve in six of our poliomyelitic fluids: 111221000.

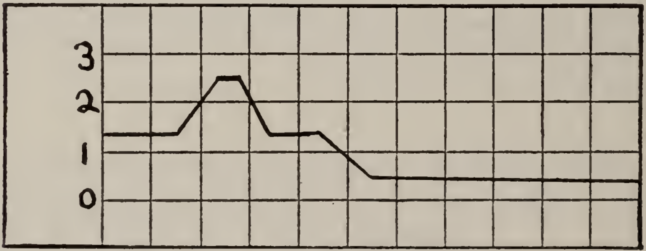

Chart 6.—Curve in five of our poliomyelitic fluids: 112110000.

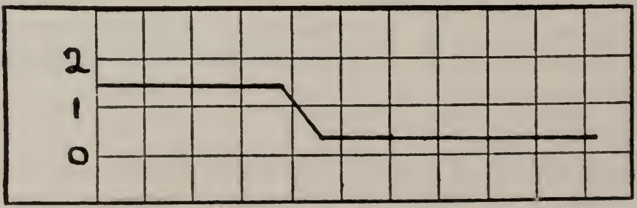

Chart 7.—Curve in fourteen of our poliomyelitic fluids: 111100000.

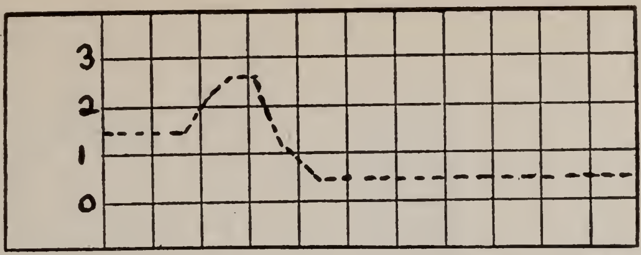

Chart 8.—Curve in four of our poliomyelitic fluids: 1121000000.

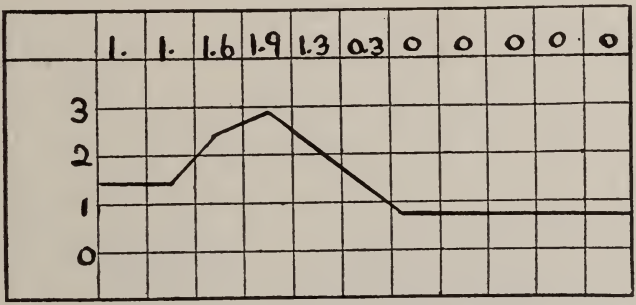

Chart 9.—Composite curve in 114 poliomyelitic fluids: 1122100000.

Every poliomyelitis fluid examined gave a curve falling in one of these six groups, or approximately so, but this cannot be taken to be of absolute diagnostic value, since a few fluids from patients with epidemic and tuberculous meningitis also fell in these groups. Of perhaps greater value is the differentiation of a poliomyelitic fluid with a practically normal chemistry from that of meningism. In only one instance was the fluid from a patient with poliomyelitis negative, even with a practically normal chemistry, while all the curves from patients with meningism have been negative even with a slightly increased chemistry. Not enough fluids from patients with meningism have been as yet examined to enable us to feel sure that the fluids from them are always negative. If this proves to be true, the gold chlorid curve will be of great value in differentiating poliomyelitis fluid with slight chemical changes from fluids of meningism.

TABLE 10.

ONE HUNDRED AND FOURTEEN POLIOMYELITIS FLUIDS EXAMINED BY GOLD CHLORID METHOD.

Seventy-seven of them fell in one of 6 curves as follows:

	No. of Cases
1122100000	40
1123210000	8
1112210000	6
1121100000	5
1111000000	14
1121000000	4

The other thirty-seven had various curves similar to the foregoing six, and are as follows:

	No. of Cases		No. of Cases		No. of Cases
1100000000	1	1221100000	2	22332100	1
1111100000	1	1222100000	2	22343210	1
1111110000	2	1223210000	3		—
1111111100	1	1222332100	1		2
1111231100	1	1232100000	2		
1112100000	4	1233100000	1		
1112110000	2	1233210000	1		
1112221000	1		—		
1112233210	1		12		
1112321000	2				
1122110000	3				
1122210000	2				
1123321000	1				
1124432100	1				
	—				
	23				
	12				
	2				
	—				
	37				

Normal or meningism curve	0000000000 or 1110000000
Curve of 114 poliomyelitis fluids	1122100000
Curve of 13 tuberculous fluids	1112221000
Curve of 15 epidemic cerebrospinal meningitis	1111233210

2. Quantitative Studies in Spinal Fluids

JOSEPHINE B. NEAL and R. L. KAHN.

Qualitative studies of the albumin, globulin and glucose, with rough quantitative estimations, have always been made by the meningitis division as a part of the routine examination of spinal fluids. The need of accurate quantitative determinations, however, has been keenly felt.

In view of the more extensive use now made of spinal fluid examinations for diagnosis, it would seem desirable to obtain as comprehensive a knowledge of the subject as possible. Moreover, there is always the hope and the possibility in studying such a new field, that some facts of practical value in the way of diagnosis and prognosis may be brought to light. It was, therefore, a source of great gratification to us when, in connection with the work of the epidemic of poliomyelitis in the summer of 1916, the opportunity was offered us to undertake such studies.

The determinations attempted embrace total, nonprotean, urea and ammonia nitrogen, uric acid, creatin, creatinin, sugar and cholesterol. The methods employed were the adaptations and, in some cases, modifications of the microchemical procedures used in blood investigations. These methods are described somewhat in detail at the end of the article.

With the exception of urea, which has been extensively studied by French workers, quantitative studies in spinal fluids are comparatively few, due, undoubtedly, to the fact that until recently, microchemical methods not being available, most single chemical tests required a larger amount of spinal fluid than was usually obtained at one puncture. Along all the lines of quantitative work the French have contributed most to the subject.

The work up to 1909 is reviewed by Anglada.[19] He gives various tables from different workers and concludes that the normal content of albumin in the spinal fluid is about 15 mg. per 100 c.c., and that normally, this albumin is serum-globulin, while in a true meningitis serum, albumin is also present and the total quantity is greatly increased, running as high as 300 mg. per 100 c.c. The normal urea is given as from 15 to 35 mg. per 100 c.c., and results as high as 438 mg. per 100 c.c. in uremia. The normal sugar is given as 0.045 to 0.055 per cent. He considers 0.06 to 0.10 per cent. or more, as an increase in the glucose, and cites such findings in diabetes and certain toxic and infectious conditions and diseases of the nervous system. In cases of meningitis and certain other conditions of the central nervous system, he finds a decrease in the sugar.

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[19] Anglada: Le liquide céphalorachidien, Paris, 1909.

In a very complete and extensive study of spinal fluid, Mestrezat[20] gives in normal fluids, the following findings:

Albumin Averages	18 mg. per 100 c.c.;	limit, 13 to 30 mg.
Urea averages	6 mg. per 100 c.c.;	limit, 3 to 10 mg.
Sugar averages	0.053% per 100 c.c.;	limit, 0.048 to 0.058%

In purulent meningitis the albumin is increased to 300 and even to 1,160 mg. per 100 c.c.; in tuberculous meningitis, from 100 to 300 mg. per 100 c.c.

Soper and Granat[21] studied the urea in ninety-seven spinal fluids, having in mind, especially, the relation of the urea content to the prognosis in cases of uremia. The blood urea, also, was examined in certain cases and agreed closely with the content of the spinal fluid, as had been previously stated by other workers. They found the urea varying from 10 to about 50 mg. per 100 c.c. in conditions without nephritis, though in certain cases of tuberculous meningitis it rose to 170 mg. per 100 c.c. They give a very good review of the literature, the earlier work having been done mainly by French workers.

According to Plaut, Rehm and Schottmuller,[22] normal spinal fluid contains from 30 to 60 mg. of protein per 100 c.c. These figures are increased in various diseases.

Pfeiffer, Kober and Field[23] studied a number of spinal fluids, mostly from syphilitic cases, with especial reference to the relation of the total protein, total globulin and euglobulin to the Wassermann reaction. They found the amount of protein varying from 20 to 100 mg. per 100 c.c. of fluid. Their fluids were nearly all pathologic.

Rosenbloom, [24] studying eleven fluids, found the nitrogen in spinal fluids varying from 0.172 parts per 100 in a normal fluid, to 0.362 in a case of meningitis.

Woods[25] found, in agreement with earlier workers, that the urea in the blood and spinal fluid was the same. He also observed that the total nonprotein nitrogen was about 25 per cent. lower than in the blood; that is, about 10 mg. per 100 c.c. of fluid. The urea was about 78 per cent. of the total nonprotein nitrogen; the total nonprotein nitrogen in cases of chronic nephritis varied from 17.85 to 188.69 mg. per 100 c.c., and the urea from 11.09 to 166.66 mg. per 100 c.c.

A similar relation between the nonprotein nitrogen in the spinal fluid and blood was reported earlier by Mollard and Froment.[26]

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[20] Mestrezat: Le liquide céphalorachidien normal et pathologique, Paris, 1912.

[21] Soper and Granat: The Archives Int. Med., 1914, 13, 131.

[22] Plaut, Rehm and Schottmuller: Leitfaden zur Untersuchung der zerebrospinal Flüssigkeit, Jena, 1913, p. 16.

[23] Pfieffer, Kober and Field: Proc. Soc. Exper. Biol. and Med., 1915, 12, 153.

[24] Rosenbloom: Biochem. Bull., 1916, 5, 17.

[25] Woods: The Archives Int. Med., 1915, 16, 577.

[26] Mollard and Froment: Jour. de physiol. et de path. gén., 1909, 11, 263.

The concentration of urea in the spinal fluid, however, appears to be about equal to that in the blood, as is reported by Soper and Granat,[21] Cullen and Ellis[27] and other workers, referred to in the foregoing atircles.

Fine and Myers [28] found that in nephritis the concentration of creatinin in the spinal fluid is 46 per cent., of creatin 22 per cent., of uric acid 5 per cent. of the concentrations of the respective substances in the blood.

Hopkins[29] studied the sugar content of the spinal fluid, comparing it in certain cases with the blood sugar, which he found to be, in normal conditions, about 10 mg. per 100 c.c. higher than in the spinal fluid. He determined the sugar content in 141 cases, including many conditions—meningitis, diabetes, infections, intoxications, epilepsy, nephritis, nervous diseases and syphilis. The earlier work along this line is reviewed.

Schloss and Schroeder[30] determined the sugar content of a large number of spinal fluids, both normal and pathologic, from infants and children. They concluded that the normal amount varies from 0.05 to 0.134 per cent. In tuberculous meningitis it varied from an amount too small to be determined to normal. In poliomyelitis it was normal in three cases and decreased in a fourth. In epidemic meningitis it was generally decreased at the height of the disease and increased with recovery, a fact previously noted by Connall[31] and by DuBois and Neal.[32] Kraus and Corneille[33] studied the sugar content in the spinal fluid in both normal and pathologic conditions. They found the range for normal fluids to be between 0.045 and 0.110 per cent. They also found a rise in the sugar content in recovering cases of epidemic cerebrospinal meningitis and a decrease in fatal cases.

The presence of cholesterol in spinal fluids has been reported by various workers[34] in different forms of paralysis.

In this laboratory the total nitrogen in poliomyelitis was found to be in the neighborhood of 25 mg. per 100 c.c. of spinal fluid. In various forms of meningitis, the total nitrogen was found to be considerably increased, extending from 35 mg. to about 150 mg. per 100 c.c. Total nitrogen determinations alone, however, are of comparatively little value in view of the variations of the nonprotein nitrogen content. This it is perhaps well to emphasize, in view of the attempt of Landau and Halpern[35] to show that a certain antagonism exists between the total nitrogen and chlorids in spinal fluids; that a high finding of the one corresponds to a low finding of the other. A high total nitrogen may often be due to an increase in the nonprotein nitrogen, particularly in the presence of kidney disturbances.

The nonprotein nitrogen content in normal spinal fluid is about 50 to 70 per cent. of the total nitrogen, and urea about 60 to 80 per cent. of the nonprotein nitrogen. In pathologic conditions these relations do not hold.

The determinations of ammonia nitrogen were unsatisfactory, because sufficiently large quantities of a single fluid required for a test were not available. Mixed fluids, therefore, had to be resorted to. This procedure seemed justifiable in view of the fact that Frenkel-Heiden[36] was unable to demonstrate ammonia in spinal fluids. The findings show wide variation, with figures ranging from 0.1 mg. to 0.9 mg. of ammonia nitrogen per 100 c.c. of spinal fluid in poliomyelitis. It might be said, also, that these fluids, although sterile at the time of the determinations, had been kept in the icebox for several weeks, which might slightly affect the results.

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[27] Cullen and Ellis: Jour. Biol. Chem., 1915, 20, 511.
[28] Fine and Myers: Proc. Soc. Exper. Biol. and Med., 1916, 13, 70.
[29] Hopkins: Am. Jour. Med. Sc., 1915, 150, 847.
[30] Schloss and Schroeder: Am. Jour. Dis. Child., 1916, 11, 1,
[31] Connall: Quart. Jour. Med., 1909-1910, No. 3, p. 152.
[32] DuBois and Neal: Am. Jour. Dis. Child., 1015, 9, 1.
[33] Kraus and Corneille: Jour. Lab. and Clin. Med., 1916, 1, No. 9.
[34] Pighina: Ztschr. f. Phys. Chem., 1909, 61, 508.
[35] Landau and Halpern: Biochem. Ztschr., 1908, 9, 72.
[36] Frenkel-Heiden: Biochem. Ztschr., 1906, 1907, 2, 188.

The determinations of uric acid also were carried out on mixed fluids. The results indicate that there are present from 0.25 mg. to 0.50 mg. of uric acid per 100 c.c. of fluid in poliomyelitis.

The quantity of creatinin in poliomyelitis fluid was found to be about 0.5 mg. per 100 c.c., and somewhat less than this amount of creatin. The recent conclusions of Gettler and Baker [37] that normal blood contains no more than 0.5 mg. per 100 c.c. of creatinin is of interest in this connection. If the method of Folin and Denis be correct, then, like urea, creatinin is equally distributed in the blood and spinal fluid.

The sugar content in poliomyelitis fluids is about normal, 0.06 per cent. In meningitis the findings are usually lower.

An attempt was made to determine cholesterol in spinal fluids by the method recently suggested by Bloor.[38] In no case, however, were the amounts sufficient for quantitative determinations. All fluids tested showed the presence of traces only.

The results in detail are given in the accompanying tables.[39]

METHODS.

Total Nitrogen.—The method employed for the determination of total nitrogen in spinal fluids was a modification of the direct nesslerization method recently developed by Folin and Denis.[40] The modification in detail is presented in another place.[41] In this connection, only a brief outline of the procedure will be given.

Procedure: Two c.c. of spinal fluid are pipetted into a test tube, 1 c.c. of the concentrated acid mixture (containing 1 volume concentrated sulphuric acid, 3 volumes concentrated phosphoric acid and 1/15 volume of a 10 per cent. solution of copper sulphate) is added and the digestion carried out over a microburner until the appearance of white sulphuric acid fumes. The mouth of the test tube is then covered with a watch glass and heating continued for about a minute. The color obtained is usually straw yellow. After permitting the test tube to cool, the contents are rinsed quantitatively into a 100 c.c. volumetric flask, using about 60 c.c. of water in the process. A quantity of 10 per cent. sodium hydroxid is then added sufficient to neutralize the concentrated acid and permit a surplus of 2 c.c. of alkali, and the mixture filtered. Into another 100 c.c. volumetric flask are pipetted 1 c.c. of the concentrated acid mixture, diluted, 20 c.c. of the standard ammonium sulphate solution containing 1 mg. ammonia nitrogen and an amount of 10 per cent. alkali solution equal to that added to the unknown. This is also made up to volume, shaken and filtered.

Aliquot portions of these filtrates are employed for nesslerization. Thus 50 c.c. of the water-clear filtrates of both the unknown and standard are pipetted into two 100 c.c. volumetric flasks, diluted to about 75 c.c. with water, and 10 c.c. of Nessler's solution added to each, made up to volume, shaken and the colors compared on the colorimeter. The same results could be obtained by using 50 c.c. volumetric flasks and employing 25 c.c. quantities of respective filtrates for nesslerization.

Nonprotein Nitrogen.—Procedure: To 5 c.c. of spinal fluid in a large test tube are added, quantitatively, 2 c.c. of a freshly prepared 25 per cent. solution of glacial phosphoric acid. The test tube is then stoppered, shaken and permitted to stand one to twenty-four hours and filtered through a small dry filter paper. Either 5 or 3 c.c. quantities of the water-clear filtrates are used for a nitrogen determination, the procedure being identical with that described for total nitrogen.

Urea Nitrogen.—Procedure: To 5 c.c. of spinal fluid in a 100 c.c. volumetric flask are added about 5 c.c. of water and 0.1 gm. of dry urease, shaken and permitted to

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37. Gettler and Baker: Jour. Biol. Chem., 1916, 25, 211.
38. Bloor: Jour Biol. Chem., 1916, 24, 227.
39. Dr. Kahn was assisted by Mr. Benjamin Shapiro in the technical work.
40. Folin and Denis: Jour. Biol. Chem., 1916, 26, 473.
41. Kahn: Jour. Biol. Chem., 1916, 28, 203.

stand at room temperature from twenty to forty minutes. This is then diluted with about 50 c.c. of water, 2 c.c. of freshly prepared glacial phosphoric acid added, also 0.5 gm. of Merck's charcoal, and made up to volume. This is shaken from time to time and allowed to stand for forty-five minutes or more, when it is ready to be filtered. Definite portions of the water-clear filtrates are used for nesslerization, as in the cases of the total and nonprotein nitrogen determinations.

Ammonia Nitrogen.—Procedure: To 25 c.c. spinal fluid are added 3 c.c. of glacial phosphoric acid, mixed, allowed to stand about an hour and filtered; 25 c.c. of the filtrate are pipetted into a 50 c.c. volumetric flask, 5 c.c. Nessler's solution added, made up to volume and compared with a standard ammonium sulphate solution containing 0.25 mg. nitrogen in 100 c.c.

Uric Acid.—Procedure: The Benedict[42] modification of the Folin and Denis[43] method was employed. Twenty-five c.c. spinal fluid are added to boiling hundredth normal acetic acid in a casserole, boiling continued for about a minute, removed from the flame, about 200 c.c. of boiling water added and poured over a folded filter. The filtrate is then concentrated to about 50 c.c., cooled, and about 0.5 c.c. of dialyzed iron added drop by drop, shaken with each addition, then filtered and the water-clear filtrate concentrated to about 2 to 3 c.c.; transferred quantitatively to a centrifuge tube, using about 5 c.c. of hot water to wash out the vessel. To the solution in the centrifuge tube is now added about 15 drops of ammonia-silver-magnesia mixture, allowed to stand for about ten minutes to permit precipitation, when it is centrifuged at a high speed for about five minutes. The supernatant fluid is poured off and excess of ammonia drawn off by inverting the tube on filter paper; 2 drops of a 5 per cent. solution of KCN are added to dissolve the precipitate, also 1 c.c. of the phosphotungstic acid reagent and about 8 c.c. of saturated solution of sodium carbonate. This is permitted to stand for a minute, transferred into a 25 c.c. or 50 c.c. volumetric flask, depending on the intensity of the color, made up to volume with water and compared with the standard on the Duboscq colorimeter. The latter is prepared by pipeting 5 c.c. of the uric acid standard solution into a 50 c.c. flask, and adding 2 drops of the KCN solution, 2 c.c. of phosphotungstic acid reagent and 15 c.c. of saturated sodium carbonate, and making up to the mark with water.

Creatinin and Creatin.—Procedure: For the determinations of creatinin and creatin the Folin and Denis[44] methods were employed. These methods have recently been criticised by McCrudden and Sargent.[45] The results, nevertheless, seem worth repeating in view of the creatinin and creatin studies on blood by the same methods. To 5 c.c. of spinal fluid are added 20 c.c. of saturated solution of picric acid, filtered, and 10 c.c. quantities of the filtrate employed for creatinin and creatin determinations, respectively. The standards employed are solutions of creatinin in saturated picric acid. The color is developed by adding 0.5 c.c. of 10 per cent. sodium hydroxid solution and allowing to stand ten minutes. Several standards are prepared and the color of the unknown matched with the one which approached it closest in intensity. In the case of the creatin determinations, the standard also was autoclaved. This, it is believed, reduced the chances of error considerably.

Sugar.—This was determined by means of the Lewis and Benedict[46] method slightly modified.

Procedure: Four volumes of saturated solution of picric acid are added to 1 volume of spinal fluid, shaken and filtered. To 3 c.c. of the filtrate in a test tube graduated to the 10 c.c. mark is added 1 c.c. of saturated solution of sodium carbonate and placed in boiling water for about twenty minutes, after which it is cooled and made up to 10 c.c. with water. The standard employed is a solution of glucose (Kahlbaum) in saturated picric acid. To 3 c.c. of this solution containing 0.5 mg. of glucose is added 1 c.c. of saturated sodium carbonate solution, kept in a warterbath for about twenty minutes, cooled, made up to 10 c.c. and compared on the colorimeter with the unknown.

Seventy-five poliomyelitis fluids were tested for six substances—total nitrogen, nonprotein nitrogen, urea nitrogen, creatinin, creatin and sugar (Table 11). They showed the variations set forth in Table 12. The outcome of these seventy-five cases varied from complete recovery to death.

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[42] Benedict: Jour. Biol. Chem., 1915, 20, 629.

[43] Folin and Denis: Jour. Biol. Chem., 1912-1913, 13, 469.

[44] Folin and Denis: Jour. Biol. Chem., 1914, 17, 475.

[45] McCrudden and Sargent: Jour. Biol. Chem., 1916, 26, 527.

[46] Lewis and Benedict: Jour. Biol. Chem., 1915, 20, 61.

The percentages of the substances named found in each fluid gave no indication of the final outcome.

Table 13 shows the results of the examination of spinal fluids in cases of purulent meningococcic, streptococcic and influenzal meningitis. Table 14 shows the results of successive punctures in the cases of epidemic cerebrospinal meningitis (E. C. S. M.) of Table 13. Twenty-three successive fluids from eight cases of epidemic cerebrospinal meningitis were tested for the six substances, total nitrogen, nonprotein nitrogen, urea nitrogen, creatinin, creatin and sugar. They showed the variations set forth in Table 15.

Table 16 shows the results of the examination of the spinal fluid in seventeen cases of tuberculous meningitis. Table 17 shows the variations in the findings of the substances examined for.

Table 18 gives the results of the examination of a number of fluids in various affections, and Table 19 shows the variation in total nitrogen, nonprotein nitrogen, urea nitrogen and sugar in six normal spinal fluids which were tested.

Table 20 gives the median of the total nitrogen, nonprotein nitrogen, urea nitrogen, creatin, creatinin and sugar in the various diseases. The median is used instead of the average, as it is the truer index of the greater incidence of values. It is obvious in estimating averages that a few very high or very low determinations will unduly influence the result.

TABLE 11.

RESULTS OF SPINAL FLUID EXAMINATIONS IN POLIOMYELITIS

No.	Case	Quantitative Determinations						Qualitative Determinations*			Outcome
		Total N.	Non-protein N.	Urea N.	Creat-inin.	Crea-tin.	Sugar.	Albu-min.	Globu-lin/	Feh-ling's.	
2482a†	Polio.	.....	.....	.....	0.515	0.405	0.084	±	±	+++	Recovered
2516a	Polio.	.....	.....	.....	0.500	0.449	0.082	++	+++	+++	Both legs paralyzed
2535a	Polio.	.....	.....	.....	0.492	0.445	0.098	+	+	+++	O. K.
2517a	Polio.	.....	.....	.....	0.465	0.417	0.080	+	+	+++	8 weeks; slight weakness of arms and hand
2518a	Polio.	.....	.....	.....	0.454	0.495	0.092	+	+	+++	Recovered
2682a	Polio.	.....	.....	.....	.....	.....	0.088	+	+	+++	
2662a	Polio.	.....	.....	.....	.....	.....	0.059	+	+	++|	
2542a	Polio.	.....	.....	.....	.....	.....	0.053	++	++	+++	Recovered
2618a	Polio.	.....	.....	.....	.....	.....	0.072	++	+|	+++	Recovered
2685a	Polio.	.....	.....	.....	.....	.....	0.100	++|	++|	+++	Died
2792a	Polio.	.....	.....	.....	.....	.....	0.061	+	+	+++	No paralysis
2841a	Polio.	.....	.....	.....	.....	.....	0.087	+	+sl.	+++	Recovered
2884a	Polio.	.....	.....	.....	.....	.....	0.055	+|	++	+++	4 weeks; weak back; paralysis of lower extremities
2895a	Polio.	.....	.....	.....	.....	.....	0.025	+|	+	+++	12 weeks; weakness of leg
2916a	Polio.	.....	.....	.....	0.463	0.190	0.0602	+	+	+++	Recovered
3096a	Polio.	.....	.....	.....	0.273	.....	Traces	+sl.	+	++	O. K.
3101a	Polio.	.....	.....	.....	.....	.....	0.0742	+sl.	+	++|	O. K.
3092a	Polio.	.....	.....	.....	0.560	.....	0.0742	+sl.	+|	+++	Died
3094a	Polio.	.....	.....	.....	0.360	.....	0.0523	+	+	++|	
3196a	Polio.	.....	.....	.....	0.388	0.319	0.0753	+sl.	+sl.	+++	Left deltoid; left facial paralysis
3197a	Polio.	.....	.....	.....	0.400	0.314	0.0781	++	++	+++	Died
3236b	Polio.	.....	.....	.....	0.387	0.448	0.0512	+	+|	+++	Weak left lower; brace
3241a	Polio.	25.00	20.95	15.68	0.400	0.314	0.0508	+	+	+++	8 weeks; double pelvic brace
3242b	Polio.	22.87	.....	6.93	0.350	0.350	0.060	+	+	+++	Weak right arm
3245b	Polio.	.....	.....	.....	0.326	.....	0.054	+++	+++	+++	Paralysis lower extremity
2994b	Polio.	.....	.....	.....	0.609	.....	0.0613	+	++	+++	Died
2989b	Polio.	.....	.....	.....	0.378	.....	Traces	+|	+|	++	Died
3007a	Polio.	.....	.....	.....	0.403	.....	.....	+	+	+++	Recovered
2998b	Polio.	.....	.....	.....	0.323	.....	0.0595	+	+	+++	Died
2979a	Polio.	.....	.....	.....	0.454	.....	0.0454	+sl.	+	+++	
3139a	Polio.	.....	.....	.....	.....	.....	0.1063	+	+	+++	Died
3140a	Polio.	.....	.....	.....	.....	.....	0.0552	+	+	+++	Died
3109a	Polio.	.....	.....	.....	.....	.....	0.0588	+	+	+++	
3274b	Polio.	.....	.....	.....	0.396	.....	0.0810	+	+	+++	No definite paralysis
3196b	Polio.	.....	.....	.....	0.429	.....	0.0824	+	+	+++	Left deltoid; left facial paralysis
3274a	Polio.	.....	.....	.....	.....	.....	0.0641	+	+	+++	No definite paralysis
3207a	Polio.	.....	.....	15.25	.....	.....	.....	++	++	+++	Weak right deltoid
3252a	Polio.	17.33	.....	.....	.....	.....	.....	+	+	+++	O. K.

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* The + marks in this column in all tables indicate the albumin, globulin and reduction of Fehling's solution, respectively.

† Successive fluids from the same cases are indicated by a, b, c, etc.

TABLE 11.

RESULTS OF SPINAL FLUID EXAMINATIONS IN POLIOMYELITIS—(*Continued*)

No.	Case	Quantitative Determinations						Qualitative Determinations			Outcome
		Total N.	Non-protein N.	Urea N.	Creat-inin.	Crea-tin.	Sugar.	Albu-min.	Globu-lin/	Feh-ling's.	
3310b	Polio.	22.72	14.56	.....	.....	.....	.....	+	+	+++	8 weeks; left facial weakness
3314b	Polio.	33.30	.....	.....	.....	.....	.....	++	++	+++	Unable to sit up; left post. splint
3315c	Polio.	25.85	.....	6.62	.....	.....	.....	+	+	+++	8 wks.; weak lowers; slight weakness of uppers
3300a	Polio.	21.00	11.0(?)	.....	.....	.....	.....	+	+	+++	8 weeks; relaxed abdominal muscles
3307b	Polio.	24.25	.....	12.50	.....	.....	.....	++	+	+++	Recovered
3322b	Polio.	.....	.....	12.50	.....	.....	.....	++	++	+++	8 weeks; left facial paralysis
3327c	Polio.	.....	13.72	.....	.....	.....	.....	++++	++++	++	O. K.
3324b	Polio.	.....	16.34	.....	.....	.....	.....	++++	++++	+++	Left deltoid weakness
3331a	Polio.	34.00	20.66	5.35	.....	.....	.....	++	+	+++	Died
3341b	Polio.	30.00	.....	18.51	.....	.....	.....	++	++	+++	O. K.
3342b	Polio.	.....	12.13	.....	.....	.....	.....	+	+	+++	No definite paralysis
3343b	Polio.	.....	12.08	.....	.....	.....	.....	+sl.	+	++++	No paralysis noted
3354a	Polio.	.....	.....	11.36	.....	.....	.....	++++	++++	+++	No definite paralysis; weak gluteals
3357b	Polio.	22.57	16.64	.....	.....	.....	.....	++	+	+++	
3359c	Polio.	26.25	.....	.....	.....	.....	.....	+	+	++++	Weak right gluteals and right lumbar muscles
3196d	Polio.	19.50	15.54	13.12	.....	.....	.....	+	++	+++	Left deltoid; left facial paralysis
3358d	Polio.	.....	14.01	.....	.....	.....	.....	++	+	+++	8 wks.; posterior splints
3361c	Polio.	.....	17.87	.....	.....	.....	.....	++	++	+++	Died
3310c	Polio.	.....	.....	9.61	.....	.....	.....	+	++	+++	8 wks.; weak left quadriceps extensor
3376c	Polio.	18.42	.....	.....	.....	.....	.....	+	+sl.	+++	O. K.
3300b	Polio.	20.61	.....	7.81	.....	.....	.....	+	+	+++	Relaxed abdominal muscles
3322c	Polio.	.....	17.85	.....	.....	.....	.....	++	++	+++	Paralysis of left facial; 8 weeks
3379b	Polio.	16.87	.....	.....	.....	.....	.....	+	+	+++	8 wks.; slight facial paralysis; slight weakness of both quadriceps extensors
3384a	Polio.	20.00	.....	.....	.....	.....	.....	++	++	+++	Died
3324c	Polio.	20.68	.....	.....	.....	.....	.....	++	++	+++	
3274c	Polio.	21.42	.....	.....	.....	.....	.....	++	++	+++	No definite paralysis
3397a	Polio.	16.37	13.72	8.83	.....	.....	.....	+.	+sl.	+++	8 weeks; weak thigh and back muscles
3392a	Polio.	17.48	.....	5.06	.....	.....	.....	+	+	+++	Right arm and leg paralysis
3410c	Polio.	.....	.....	20.75	.....	.....	.....	+	+	+++	Paralysis left quadriceps extensor
3341c	Polio.	.....	15.88	.....	.....	.....	.....	+	+	+++	O. K.
3412c	Polio.	22.62	.....	.....	.....	.....	.....	++	+++	+++	Right deltoid
3436a	Polio.	24.62	.....	14.62	.....	.....	.....	++	++	+++	Died
3439a	Polio.	.....	24.78	.....	.....	.....	.....	++	++	+++	Died
3497a	Polio.	31.25	.....	26.6	.....	.....	.....	+	+	+++	
2842a	Polio.	(?)	.....	.....	.....	.....	0.0523	+	++	+++	
3523a	Polio.	16.87	8.91	.....	0.528	.....	0.0943	+	+	+++	

TABLE 12.

FINDINGS IN SUCCESSIVE PUNCTURES

	Number of Fluids	Variation per 100 c.c., Mg.
Total nitrogen	24	16.37 to 34.00
Nonprotein nitrogen	16	8.91 to 24.78
Urea nitrogen	17	5.06 to 26.6
Creatinin	22	0.273 to 0.609
Creatin	11	0.190 to 0.495
Sugar	36	0.025 to 0.1063

TABLE 13.

RESULTS OF EXAMINATION OF SPINAL FLUIDS IN PURULENT MENINGITIS

No.	Case	Quantitative Determinations						Qualitative Determinations			Outcome.
		Total N.	Nonprotein N.	Urea N.	Creatinin.	Creatin.	Sugar.	Albumin.	Globulin.	Fehling's.	
3017a	Epidemic cerebrospinal meningitis.	.....	.....	.....	0.595	.....	Traces	++++	++++	++	O. K.
3225a	Epidemic cerebrospinal meningitis.	.....	.....	.....	0.326	.....	Traces	+++	+++	—	O. K.
3222c	Epidemic cerebrospinal meningitis.	45.15	.....	14.12	.....	.....	.....	+++	+++	+++	O. K.
3268a	Epidemic cerebrospinal meningitis.	34.25	15.86	.....	.....	.....	.....	++	++	+	O. K.
3302b	Epidemic cerebrospinal meningitis.	62.20	.....	10.25	.....	.....	.....	++++	++++	+	Died
3363a	Epidemic cerebrospinal meningitis.	24.46	.....	.....	.....	.....	.....	++++	++++	++	O. K.
3386a	Epidemic cerebrospinal meningitis.	39.80	.....	.....	.....	.....	.....	+++	++++	±	Died
3442a	Influenzal meningitis	40.25	.....	6.28	.....	.....	.....	++++	++++	—	Died
3468c	Influenzal meningitis	138.75	.....	.....	.....	.....	.....	++++	++++	—	Died
3472a	Influenzal meningitis	51.50	.....	.....	.....	.....	.....	++++	++++	±	Died
3500a	Streptococcus meningitis	86.30	.....	11.10	.....	.....	.....	++++	++++	—	Died
3541a	Epidemic cerebrospinal meningitis.	69.50	46.66	15.15	67.1*	21.7†	Traces	++++	++++	—	
3574a	Epidemic cerebrospinal meningitis.	.....	.....	.....	.....	.....	Traces	+++	+++	—	O. K.
3545b	Streptococcus meningitis	23.35	18.08	.....	77.4*	.....	0.075	++++	++++	—	

* Per cent. of nonprotein nitrogen in total.
† Per cent. of urea in total.

TABLE 14.

RESULTS OF SUCCESSIVE PUNCTURES IN CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS

No.	Quantitative Determinations.						Quantitative Determinations.			Outcome.
	Total N.	Non-protein N.	Urea N.	Per Cent. of N. P. N. in Total.	Per Cent. Urea in Total.	Sugar.	Albumin.	Globulin.	Fehling's.	
3441										
2d puncture...	120.0	33.47	21.62	.....	.....	.....	++++	++++	+	O. K.
5th puncture...	36.75	.....	.....	.....	.....	.....	+++	+++	++	
3473										
1st puncture...	50.00	.....	.....	.....	.....	.....	++++	++++	—	O. K.
2d puncture...	53.75	.....	.....	.....	.....	.....	++++	++++	+	
4th puncture...	47.15	26.62	9.20	.....	.....	.....	++++	++++	+	
5th puncture...	43.45	21.93	11.60	.....	.....	.....	++++	++++	+++	
3487										
1st puncture...	95.00	45.83	36.48	.....	.....	.....	++++	++++	—	Died
2d puncture...	250.00	136.25	52.28	.....	.....	.....	++++	++++	—	
3044										
4th puncture...	73.50	26.60	.....	0.535*	0.704†	Traces	++++	++++	±	Died
5th puncture...	.....	.....	.....	0.476*	.....	Traces	++++	++++	±	
6th puncture...	.....	.....	.....	0.476*	.....	0.0628	++++	++++	±	
3512										
1st puncture...	53.50	25.47	.....	47.6	.....	Traces	++++	++++	—	Died
4th puncture...	29.37	13.62	.....	46.3	.....	Traces	++++	++++	—	
5th puncture...	43.25	15.00	.....	34.6	.....	.....	++++	++++	—	
6th puncture...	47.35	19.55	6.14	41.2	12.9	.....	++++	++++	—	
7th puncture...	40.53	18.25	7.72	45.0	19.0	Traces	++++	++++	—	
8th puncture...	48.50	19.55	7.54	40.3	15.5	Traces	++++	++++	—	
9th puncture...	128.00	31.36	9.25	24.5	7.2	Traces	++++	++++	—	
3530										
1st puncture...	52.00	21.85	10.41	42.0	20.0	Traces	++++	++++	—	O. K.
2d puncture...	.....	.....	.....	.....	.....	.....	++++	++++	—	
3525										
1st puncture...	36.10	20.58	10.25	57.0	28.3	Traces	++++	++++	—	O. K.
2d puncture...	39.25	22.94	6.38	58.4	16.2	Traces	++++	++++	—	
3d puncture...	46.25	21.77	9.25	47.0	20.0	Traces	++++	++++	—	
4th puncture...	.....	.....	6.87	.....	.....	Traces	++++	++++	—	
5th puncture...	71.00	25.69	9.61	36.1	13.5	Traces	++++	++++	—	
6th puncture...	45.45	20.63	5.43	45.3	11.7	Traces	++++	++++	—	
7th puncture...	86.00	27.36	9.85	31.8	11.4	Traces	++++	++++	—	
8th puncture...	.....	.....	6.43	.....	.....	Traces	++++	++++	—	
13th puncture...	.....	.....	9.47	.....	.....	.....	++++	++++	+++	
3551										
1st puncture...	22.72	13.00	5.11	57.2	22.4	Traces	++++	++++	—	O. K.
3d puncture...	.....	.....	3.66	.....	.....	Traces	+++	++	++	
4th puncture...	.....	.....	6.77	.....	.....	.....	+++	+++	+	
5th puncture...	.....	.....	4.55	.....	.....	.....	+++	+++	+++	

* Creatinin. † Creatin.

TABLE 15.

RESULTS OF TESTS OF TWENTY-THREE SUCCESSIVE FLUIDS FROM EIGHT CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS SHOWING VARIATIONS.

	Number of Fluids.	Variations per 100 c.c. Mg.
Total nitrogen	9	22.72 to 250.00
Nonprotein nitrogen	9	13.00 to 136.25
Urea nitrogen	9	3.66 to 53.28
Creatinin	9	0.326 to 0.595
Creatin	9	0.704 to ....
Sugar	9	Traces to 0.0628
Per cent. nonprotein nitrogen in total	15	24.5 to 58.4
Per cent. urea nitrogen in total	15	7.2 to 28.3

If the termination was favorable, nitrogen, nonprotein nitrogen and urea were decreased; sugar was increased; if the termination was unfavorable, opposite results were obtained.

TABLE 16.

RESULTS OF TESTS OF CEREBROSPINAL FLUIDS IN TUBERCULOUS MENINGITIS.

No.	Case	Quantitative Determinations.						Qualitative Determinations			Outcome.
		Total N	Nonprotein N	Urea N	Creatinin	Creatin	Sugar	Ablumin	Globulin	Fehling's	
2508b	Tuberculous meningitis	....	....	....	0.487	0.563	0.060	++	+++	+++	Died
2401a	Tuberculous meningitis	....	....	....	0.765	0.735	0.028	+++	+++	+	
3115a	Tuberculous meningitis	....	....	....	....	....	Traces	++	++	+sl.	Died
2966a	Tuberculous meningitis	....	....	....	....	....	Traces	++	++|	++|	Died
3284a	Tuberculous meningitis	34.50	....	6.66	....	....	....	++	+	++	Died
3333a	Tuberculous meningitis	34.40	....	....	....	....	....	++++	++++	+	
3339b	Tuberculous meningitis	29.70	17.25	7.93	....	....	....	++++	++++	+++	Died
3335a	Tuberculous meningitis	42.50	....	14.18	....	....	....	++++	++++	–	
3434a	Tuberculous meningitis	24.35	....	11.50	....	....	....	++	++	+	Died
3304c	Tuberculous meningitis	26.50	13.88	7.06	....	....	....	+++	+++ (See 3284)	++	Died
3469a	Tuberculous meningitis	25.25	15.44	....	....	....	....	++++	++++	++	Died
3478b	Tuberculous meningitis	20.86	....	....	....	....	....	++++	++++	++	Died
3514a	Tuberculous meningitis(?)	16.3	9.05	3.13	55.5*	19.2†	....	++	++	–	
3520a	Tuberculous meningitis	23.35	12.82	....	54.9*	....	Traces	++++	++++	–	
3547a	Tuberculous meningitis	22.72	....	....	....	....	Traces	++++	++++	–	
3583a	Tuberculous meningitis	....	....	7.22	....	....	....	++++	++++	+	
3584a	Tuberculous meningitis	....	....	4.54	....	....	....	++++	++++	+	

* Per cent. of nonprotein nitrogen in total.
† Per cent. of urea in total.

TABLE 17

SHOWING VARIATIONS IN THE FINDINGS IN SEVENTEEN CASES OF TUBERCULOUS MENINGITIS.

	Variations per 100 c.c., Mg.
Total nitrogen	20.86 to 34.50
Nonprotein nitrogen	12.82 to 17.25
Urea nitrogen	4.54 to 14.18
Creatinin	0.487 to 0.7652
Creatin	0.563 to 0.735
Sugar	Traces to 0.060

TABLE 18.

RESULTS OF EXAMINATION OF MISCELLANEOUS FLUIDS.

No.	Case.	Quantitative Determinations.						Qualitative Determinations.			Outcome.
		Total N.	Non-protein N.	Urea N.	Creatinin.	Creatin.	Sugar	Albumin.	Globulin.	Fehling's.	
2821a	Meningism; typhoid	....	....	....	....	....	0.066	+	+	+++	
3095a	Meningism; endocarditis	....	....	....	0.365	....	0.0806	+	+	+++	Died
3113b	Meningism; intestinal trouble	....	....	....	....	....	0.0824	+sl.	+sl.	+++	O. K.
3244a	Possible brain tumor	....	....	....	0.350	....	0.051	+++	+++	+++	
3138a	Rheumatism	45.25	31.06	14.81	....	....	....	+	+	+++	O.K.
?	Syphilis	23.35	19.38	....	....						
3251a	Cerebrospinal lues	....	....	....	0.373	0.377	0.048	+	+sl.	+++	Imp'vd
3240a	Normal (?)	14.35	....	....	....	....	....	+	+	+++	
3495a	Normal	15.70	13.69	....	....	....	....	±	+sl.	+++	
3515a	Meningism	10.18	....	6.46	....	63.4†	....	±	±	+++	
3516a	Meningism	15.30	13.68	....	89.4*	....	0.0888	±	±	+++	
3528a	Syphilis	19.45	14.15	....	72.7*	....	0.075	±	±	+++	
3529a	Meningism with pneumonia	9.67	....	5.35	....	55.3†	0.0923	±	±	+++	
3569a	Meningism	....	....	12.05	....	....	....	±	±	+++	
3571a	Staph. albus meningitis	....	....	....	....	....	Traces	++++	++++	±	
3593d	Meningism with pneumonia	22.72	22.38	10.00	98.5*	44.0†	0.0798	+	+	+++	

* Per cent. of nonprotein nitrogen in total.
† Per cent. of urea in total.

TABLE 19.

SHOWING VARIATIONS IN THE FINDINGS IN SIX NORMAL FLUIDS.

	Variations per 100 c.c., Mg.
Total nitrogen	9.67 to 15.70
Nonprotein nitrogen	13.68 to 13.69
Urea nitrogen	5.35 to 12.05
Sugar	0.0824 to 0.0923

TABLE 20.

MEDIAN CEREBROSPINAL FLUID FINDINGS IN VARIOUS DISEASES.

	Total Nitrogen, mg. per 100 c.c.	Nonprotein Nitrogen, mg. per 100 c.c.	Urea Nitrogen, mg. per 100 c.c.	Creatinin, mg. per 100 c.c.	Creatin, mg. per 100 c.c.	Sugar, Per Cent.
Poliomyelitis..........	22.57 25 cases	15.71 16 cases	12.50 17 cases	0.400 22 cases	0.405 11 cases	0.0613 37 cases
Epidemic cerebrospinal meningitis..........	47.25 30 cases	21.93 23 cases	9.25 26 cases	0.476 5 cases	0.704 1 case	Traces 23 cases
Tuberculous meningitis.	25.88 10 cases	13.88 5 cases	7.14 8 cases	0.626 2 cases	0.649 2 cases	Traces 6 cases
Miscellaneous.........	23.04 14 cases	18.08 7 cases	10.00 7 cases	0.365 5 cases	0.377 1 case	0.075 12 cases

PART III.

Experimental Studies

H. L. Abramson

Poliomyelitis in Monkeys from Virus of the 1916 Epidemic

Landsteiner and Popper first produced poliomyelitis in monkeys in 1909. Since then workers in poliomyelitis the world over have performed similar experiments with success. This epoch-making discovery has served as an entering wedge to such workers as Landsteiner, Leviditi, Flexner and Lewis, Roemer and Joseph, Kling, Petterson and Wernstedt, and many others, who have greatly increased our knowledge of the etiologic agent of acute poliomyelitis.

The present work was instituted at the Bureau of Laboratories for the purpose of furnishing fresh material to the entire group of workers for the following studies[47]:

1. Characteristics of the virus of the 1916 epidemic.
2. Isolation and cultivation of organisms said to be the cause of acute poliomyelitis.
3. Immunity problems, active and passive.
4. Problems of transmission.

The first attempts to produce the disease in monkeys resulted in failure. On account of lack of a fresh supply of monkeys two of the Rhesus variety that had been the property of the Department for several years, were used. These received heavy suspensions of material from the brain and cords of two patients that were clinically and pathologically undoubted cases of acute poliomyelitis. The animals received 0.5 c.c. of a 20 per cent. saline emulsion intracerebrally and 2 c.c. of the same suspension into the tissues around each sciatic nerve. After one month's observation, these inoculations were repeated with the glycerinated material of the same cases, but in addition they received 10 c.c. of the virus suspension intraperitoneally. These inoculations were no more successful than the first. These monkeys, 98 and 102, later received massive inoculations by three routes, intracerebral, perisciatic and intraperitoneal, of known virulent material from a monkey of the second generation. This, too, proved unsuccessful. It was decided that these monkeys were very refractory and would serve as a source of immune serum for later experiments.

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[47] Williams, A. W.: Proceed. N. Y. Path. Soc., 1917, 17, p. 15.

Brain and cord material from three new cases that were clinically positive cases of acute poliomyelitis was then inoculated by three routes, intracerebral, perisciatic and intraperitoneal, into two monkeys from a fresh supply, one being a rhesus and the other a sapajou, a South American ring tail monkey. They received 0.5 c.c., 2 c.c. and 10 c.c. of a 20 per cent. suspension of this material, respectively, in the three ways above mentioned.

On the seventh day after the inoculation, Monkey 1, *Macacus rhesus,* presented tremor of the head and weakness in the left leg. This condition progressed to complete paralysis of all the limbs, first the left leg, then right leg, left arm, right arm, convulsions and respiratory failure. The monkey died the third day after the appearance of the muscular weakness. Monkey 2, sapajou, exhibits no symptoms at this writing, three and one-half months after inoculation. Two other animals of this variety were subsequently inoculated with a virulent virus, but these inoculations were unsuccessful also, confirming the unsuitability of this type of monkey for experimental work in the study of poliomyelitis.

No further difficulty was experienced in passing the virus obtained from Monkey 1 through a series of sixteen monkeys, fourteen of the rhesus variety and two South African mangabeys. The virus is now in the eighth generation and exhibits evidence of increased virulence.

The animals of the second and third generations were inoculated with heavy suspensions of the brain and cord material by the three ways, intracerebral, perisciatic and intraperitoneal, but subsequent animals were infected by injection of 0.5 c.c. of 10 per cent. suspensions into the brain. The last two animals received 0.5 c.c. of 5 per cent. suspension intracerebrally.

TECHNIC.

The technic followed was that in use at the laboratory for the preparation of antirabic treatments. Fresh brain and cord material was weighed and ground up in a sterile mortar and sterile salt solution added to make the desired strength. The intracerebral inoculations were made always into the right cerebral hemisphere. The animals were anesthetized. An incision was made in the middle of the scalp over the forward part of the skull. The wound was retracted to the right ride and a small trephine opening made with an awl in the right frontal bone ¼ inch anterior to the coronal suture, just to the right of the sagittal suture. The suspension was injected very slowly. It is well not to incise the pericranium, for, if intact, it acts as a valve over the site of the trephine opening and prevents the escape of the injected material. The retracted scalp when it resumes its normal position also aids in the prevention of leakage. In performing the perisciatic inoculations, care was taken to place the needle in close relation to the posterior aspect of the middle of the femur before expelling the material.

Of the seventeen animals inoculated, all but two, *Macacus rhesus* 50 and 51, exhibited frank signs of paralysis. On the fourth day after the inoculation, No. 50 became quite subdued and huddled in a corner of the cage. On being forced to move, it was noticed that he limped slightly on the left hind leg. This animal then received two intraspinal injections of serum obtained from *Macacus rhesus* 102 on successive days. The animal seemed to improve rapidly, and at this date shows no weakness in any limb.

Macacus rhesus 51 exhibited symptoms of distress on the fourth day after inoculation, which consisted of tremors and general weakness; but showed no evidence of paralysis in any limb. This general muscular weakness progressed and the monkey died on the third day after onset of symptoms. The brain showed hyperemia, and sections of the cord presented redness and swelling of the gray matter. The lungs and other organs were apparently normal. This form of the disease in monkeys has been encountered many times by workers in this field and has been called the marantic type of monkey poliomyelitis. Subsequent inoculations with material from this animal produced the typical spinal type of paralysis.

The incubation period in this series was from four to thirteen days. Eight, or 47 per cent., developed paralysis on the seventh day; two on the eighth day; two on the fourth day, and one each on the fifth, tenth, eleventh, twelfth and thirteenth days after the inoculation.

Twelve of the seventeen animals died, a mortality of 71 per cent. Of the remaining five, four exhibited residual paralyses. The average duration of illness for eleven of the twelve fatal cases was four days, the limits being two and seven days. *Macacus rhesus* 25 died twenty days after onset of paralysis. This animal had been inoculated with brain and cord material from a rabbit that had exhibited flaccid paralysis of a progressive type. This inoculation produced no effects, and after an observation period of six weeks, the monkey was again inoculated along with two other animals, 36 and 37, with material from No. 26, fourth generation. The incubation period of Nos. 36 and 37 was seven days. That of No. 25 was thirteen days. With the onset of symptoms the paralysis progressed rapidly for three days. Then the process appeared to subside and the animal improved over a period of nearly two weeks. After this quiescent period, the paralysis began where it had left off, and the animal died of respiratory failure on the twentieth day of the disease.

The character of the paralysis in all but two of the animals was that of the progressive, spinal type, beginning in the left leg, progressing to the right leg, left arm, right arm, and, in the fatal cases, to respiratory paralysis. In one animal, paralysis appeared first in the right leg, then left leg, left arm, right arm, and paralysis of respiration. In the other, the symptom appeared first in the left arm, right arm, then simultaneously in both legs and respiratory paralysis. In the nonfatal cases, except No. 50, the paralysis extended to the left arm, leaving the right arm untouched. With the cessation of the progress of the disease, the left arm recovered its power to some extent, but the paralysis of the legs was permanent.

The gross pathologic changes presented a similar appearance in all the fatal cases, consisting in moderate congestion of the pial vessels of brain and cord. Cut sections of cord at various levels presented a reddening and swelling of the gray matter, rendering it distinct and prominent.

The microscopic picture presented congestion and edema; some capillary hemorrhage; perivascular and also diffuse round cell infiltration; ganglion cells in various stages of destruction and neuronophagia; all the changes present in human cases, except that they are more severe.

Passage of this virus will be continued with the hope of increasing its virulence so as to permit us to use filtrates in further work.

Appended is a chronological table of protocols of the animal work here recorded.

Serum Therapy in Experimental Poliomyelitis

The blood of persons who have survived an attack of acute poliomyelitis contains specific substances which possess the ability to neutralize active poliomyelitis virus. This has been demonstrated by Flexner and Lewis, Roemer, Landsteiner and Leviditi, and others. It has also been shown that monkeys recovering from an attack of poliomyelitis become refractory to a second inoculation of virulent material, and in the blood of these animals can be demonstrated the neutralizing principles found in the blood of recovered human cases.

Netter[48] applied this knowledge in the treatment of a small series of human cases, with perhaps favorable results. His conclusions were not very definite. He administered, intraspinally, from 4 to 12 c.c. of serum obtained from a recovered case whose blood yielded a negative Wasserman. This serum was administered daily for four or five days, or as long as the clinical symptoms indicated its need. One case received eight injections.

Zingher,[49] of the Bureau of Laboratories, treated a larger number of patients by the intraspinal method, using serums obtained both from recovered cases of the disease and from normal persons. His report gives a favorable opinion of the treatment.

No tests were made to determine whether or not the serums obtained from the recovered patients possessed the neutralizing powers necessary to render them specific. Two cases of reinfection that came to our attention in the epidemic of the past summer may throw some doubt on the assumption that all persons recovering from one attack of the disease are immune to a second infection, or that the blood of all such persons possesses neutralizing powers.

The testing of the serum of each donor, however, for the specific principles would render the whole procedure impracticable, mainly because of the loss of time necessary to perform this test, and secondarily, because of the expense, for at the present time there is no way of demonstrating this property in serum, except by the injection into a monkey of active virus that had been placed in contact with the serum. From a practical point of view, the probabilities are that only a small percentage of the cases would give a negative test.

The clinical use of the serum of patients who have recovered from poliomyelitis is not founded on complete, clearcut animal experimentation. Its use is in part scientific, and in part empirical.

Schwarz[50] treated twenty-one patients with convalescent human serum. Of these, nine recovered without paralysis. Of a series of twenty-one other patients treated expectantly, seventeen recovered without paralysis. From this it might be inferred that the serum was of no particular advantage. Schwarz feels that too much was not to be expected of the use of the immune serum.

Prognosis in poliomyelitis is a difficult matter. Poliomyelitis is a very erratic disease and predictions as to the course it will follow in a given case are many times guess work. It will require, therefore, great numbers of cases adequately controlled to gain a true idea of the value of the serum treatment from the clinical point of view.

Flexner and Lewis[51] performed two experiments, the results of which indicate that a known specific serum may exert a prophylactic effect. An effective dose of active virus was injected intracerebrally into a monkey. Within twenty-four hours after the inoculation, and daily thereafter, the animal received intraspinal injections of immune serum for a number of days. This animal remained healthy, whereas the control died. Another monkey was inoculated by intranasal scarification with a potent virus. This animal received intraspinal injections of immune serum within twenty-four hours after the inoculation, and at three-day intervals for a number of injections. This monkey did not exhibit any symptoms of poliomyelitis, while the control died of the disease. The authors state that if the quantity of virus injected is not in excess of a certain amount, the procedure described will serve to protect animals injected with such a quantity of virus.

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[48] Netter: Bull. de l'Acad. de méd., 1914, 66, 525.

[49] Zingher: Arch. Pediat., 1916, 33, 872.

[50] Schwarz: Arch. Pediat., 1916, 33, 859.

[51] Flexner and Lewis: Jour. Am. Med. Assn., 1910, 54, 1780, and 55, 662.

The conditions of these experiments, however, do not parallel those which are met with when dealing with actual human cases, in which the virus has already become established and is multiplying in the central nervous system, as evidenced by the symptoms of the disease. In this instance the specific serum no longer can prevent; it must cure. It must counteract and nullify virus that has already become parasitic and that may have increased in amount, perhaps in excess of that which can be taken care of by injections of known immune serum.

The present work in experimental serum therapy was undertaken in an attempt to supply the conditions that are met with in actual practice. This work comprises six experiments in each of which were used two animals. One animal was serum-treated and the other acted as a control. The virus was emulsion of brain and cord material derived from the epidemic of the past summer. Virus of the second generation was used in the first series, and the virus of each succeeding generation was used in the remaining experiments. The amount of virus injected was not determined from very much previous experience with this strain. That the dosage was not excessive is evidenced by the fact that two of the control animals, 37 and 54, survived the disease, as did also two other animals, 23 and 24, which received the same amounts of virus, but were not used in these experiments.

The serum used in these experiments was obtained by heart-puncture from Monkeys 98 and 102. Both of these animals were resistant to the effects of three inoculations of brain and cord material. This material was inoculated in heavy suspensions intracerebrally, in the tissues about the sciatic nerves and in the peritoneum. The material for the first two inoculations was derived from two human cases, clinically and pathologically positive cases of poliomyelitis. The third inoculation was with material of the second generation monkey virus. These inoculations were given three weeks apart. It was assumed that as a result of these three inoculations, these monkeys were immune and that the blood of these animals ought to contain the specific antibodies. No neutralization test was performed on the serums used in this set of experiments. This, in a measure, parallels the clinical use of serum by Netter and Zingher.

In a series of experiments now under way we shall have the opportunity of observing the effects of known monkey and human immune serum in the experimental serum therapy of monkey poliomyelitis.

Method of Injection.—The serum was injected intraspinally by syringe in doses of 2 to 3.5 c.c., depending on the size of the animal and resistance offered to the plunger of the syringe. As much spinal fluid as possible was withdrawn, usually with the aid of the syringe. The first tap yielded up to 2 c.c. of slightly turbid fluid, but subsequent punctures yielded from 0.5 to 1 c.c. of fluid. The fluids, on being examined, when the quantity was sufficient, gave a globulin test and a definite increase in cells; in one instance, 690 cells per cubic millimeter. The serum was injected very slowly and with extreme caution against using any excess pressure. Only one animal exhibited severe effects after the intraspinal injection. These symptoms consisted of marked rigidity of limbs, retracted neck and labored and rapid respiration. They were, however, very transient and within a half hour after the injection the monkey appeared as before the injection. Retraction of the neck was noted after many of the injections, particularly after the symptoms of paralysis had appeared.

Time of Injection.—The time period elapsing between the injection of the virus and the beginning of the intraspinal serum injection was so arranged as to be applicable to conditions met with in actual practice. In Experiments 1 and 2 the serum treatment was begun on the first day of the appearance of any muscular weakness. In the remaining four experiments serum treatment was instituted before any symptoms had appeared, corresponding to the preparalytic stage in the human disease.

The protocols are as follows:

Experiment 1.—Sept. 1, 1916. M. rhesus 50, injected with 0.5 c.c., intracerebrally, 2 c.c. perisciatic, and 10 c.c intraperitoneally, of 10 per cent. suspension Generation II virus. M. rhesus 51, control, received 0.5 c.c., intracerebrally, of the same emulsion.

Sept. 6, 1916, No. 50 appeared sick and limped on left leg. Received 3.5 c.c. Serum 102, intraspinally. Sept. 7, 1916, 3.5 c.c. Serum 102, intraspinally. Limp still present. September 10 the limp was not noticeable and the animal appeared strong and healthy.

Sept. 5, 1916, M. rhesus 51, control, exhibited general tremors and marked muscular weakness; no definite paralysis. Died Sept. 8, 1916. Congestion of pia and swelling and reddening of gray matter of cord. The virus of this monkey subsequently produced flaccid paralysis.

Whether or not M. rhesus 50 really had poliomyelitis, it is difficult to judge. He did not at any time present frank paralysis and his rather rapid recovery is suspicious. This animal was one of whose past history we knew nothing.

Experiment 2.—Sept. 21, 1916, Rhesus 27, 0.5 c.c. intracerebrally and 2 c.c. perisciatic, of 10 per cent. virus, Generation III; Rhesus 26, control, 0.5 c.c. intracerebrally, same virus.

Sept. 28, 1916, Rhesus 27; weakness left leg; 2.5 c.c. Serum 102 intraspinally. Rhesus 26, control; paralysis left, and weakness right leg.

Sept. 29, 1916, Rhesus 27, paralysis both legs; weakness left arm; 2.5 c.c. Serum 102, intraspinally. Rhesus 26, paralysis both legs; left arm: weakness right arm; difficulty in breathing.

Sept. 30, 1916, Rhesus 27, complete paralysis of limbs and diaphragmatic breathing. Died. Rhesus 26, respiratory failure. Died.

The incubation period in both of the animals was eight days and duration of illness three days.

Experiment 3.—Oct. 2, 1916, Rhesus 36, 0.5 c.c. 10 per cent. virus, Generation IV, intracerebrally. Rhesus 37, control, 0.5 c.c. same suspension, intracerebrally.

Oct. 7, 1916. Rhesus 36, apparently normal; 3 c.c. Serum 102, intraspinally. Rhesus 37, well.

Oct. 8, 1916, Rhesus 36, well; 3 c.c. Serum 102. Rhesus 37, O. K.

Oct. 9, 1916, Rhesus 36, paralysis both legs; weakness left arm; 2.5 c.c. Serum 102. Rhesus 37, control; paralysis left leg; weakness right leg.

Oct. 10, 1916, Rhesus 36, complete paralysis both arms and legs; breathing with difficulty; no serum; condition bad. Rhesus 37, paralysis both legs; weakness left arm; does not appear as sick as 36.

Oct. 12, 1916, Rhesus 36, died of respiratory paralysis. Rhesus 37, paralysis of both legs; weakness left arm; cessation of progress of paralysis.

Nov. 29, 1916, Rhesus 37, alive; permanent paralysis in legs; left arm O. K.

In this experiment Rhesus 36 received serum treatment on the fifth day after injection of virus and two days before the onset of paralysis. Rhesus 37, control, exhibited paralysis on the same day as No. 36, an incubation of seven days. The serum treatment apparently had no influence on the incubation period. Furthermore, No. 36, despite three treatments, suffered a rapidly spreading, fatal form of the disease, whereas No. 37, control, survived the same injection, but with residual paralyses.

Experiment 4.—Oct. 13, 1916, Rhesus 43, 0.5 c.c., 10 per cent., Generation V virus, intracerebrally. Rhesus 42, control, a very large animal, 1 c.c. intracerebrally and 5 c.c. intraperitoneally of same virus.

Oct. 18, 1916, Rhesus 43 received intraspinally, 3 c.c. Serum 102; October 19, 2.5 c.c. same serum; October 20, 3 c.c. Serum 102; October 21, 2.5 c.c. Serum 98; October 22, 2 c.c. Serum 98. No signs of disease during this period. Rhesus 42, control, presented no symptoms.

Oct. 23, 1916, Rhesus 43, left leg paralyzed and weakness in right leg; 2 c.c. Serum 98 given intraspinally. Rhesus 42 shows no symptoms.

Oct. 24, 1916, Rhesus 43, paralysis progressing; 2.5 c.c. Serum 102 administered. Rhesus 42 apparently well.

Oct. 25, 1916, Rhesus 43, paralysis involving both arms and legs; 3 c.c. Serum 102 given. Rhesus 42, control, shows weakness in left leg.

Oct. 27, 1916, Rhesus 43, died of respiratory paralysis. Rhesus 42 presents involvement of both legs and weakness of left arm.

Oct. 31, 1916, Rhesus 42, died of respiratory paralysis.

In the foregoing experiment, the serum treated animal began to receive intraspinal injections of serum on the fifth day after the injection of the virus and five days prior to the appearance of symptoms in the same animal, and seven days before the control animal exhibited muscular weakness. Furthermore, the control animal had several times the dose of virus received by the test animal. The progress of the disease was more rapid in the serum treated animal, which terminated on the fifth day of the disease, than in the control animal, which died on the seventh day of the disease.

Experiment 5.—Nov. 2, 1916, Rhesus 52, 0.5 c.c. 10 per cent. Generation VI virus intracerebrally.

Oct. 31, 1916, Rhesus S, large animal, control, 0.5 c.c. intracerebrally and 5 c.c. intraperitoneally, of 10 per cent. Generation VI virus used on No. 52.

Nov. 8, 1916, Rhesus 52, apparently normal, received 2.5 c.c Serum 102 intraspinally. Rhesus S, control, showed paralysis of left arm.

Nov. 9, 1916, Rhesus 52, paralysis of left leg; 2.5 c.c. Serum 102. Rhesus S, paralysis of all limbs and respiratory paralysis. Died.

Nov. 10, 1916, Rhesus 52, paralysis progressing; 2.5 c.c. Serum 102.

Nov. 11, 1916, Rhesus 52, paralysis of both legs and left arm; 2 c.c. Serum 98 administered.

Nov. 13, 1916, Rhesus, 52, complete paralysis, including muscles of respiration. Died.

Though Rhesus 52 received a smaller does of virus and one injection of serum on the sixth day after the inoculation, the incubation period was seven days, whereas in Rhesus S, with larger dosage, and no serum therapy, the incubation was nine days. This animal, however, suffered a fulminating type of disease, dying on the second day after the appearance of symptoms. This was probably due to the early involvement of muscles of respiration. The serum treated animal died on the fifth day of the disease.

Experiment 6.—Nov. 10, 1916, Mangabey 53, 0.5 c.c. 5 per cent. Generation VII virus, intracerebrally. Mangabey 54, control, 0.5 c.c. 5 per cent. same virus, intracerebrally.

Nov. 15, 1916, Mangabey 53, appears well; 3 c.c. Serum 102, intraspinally.

Nov. 16, 1916, Mangabey 53, 3 c.c. Serum 102, appears normal.

Nov. 17, 1916, Mangabey 53, paralysis of left leg; no serum.

Nov. 18, 1916, Mangabey 53, paralysis progressing; 2.5 c.c. Serum 98. Mangabey 54, weakness left leg.

Nov. 19, 1916, Mangabey 53, paralysis both legs and left arm; 2 c.c. Serum 98 administered. Mangabey 54, marked weakness in left leg and slight weakness in right leg; progress of paralysis slow.

Nov. 20, 1916, Mangabey 53, lies prone; does not stir body; moves right arm weakly; tremor of head. Mangabey 54, condition about the same as before.

Nov. 22, 1916, Mangabey 53, complete paralysis. Died. Mangabey 54, weakness in legs more marked.

Nov. 25, 1916, Mangabey 54, progress of involvement slow; shows slight weakness of left arm; tremor of head.

Nov. 27, 1916, Mangabey 54, shows signs of improvement; left arm apparently stronger, as is also the right leg.

Dec. 1, 1916, Mangabey 54, improvement marked; feeds well; can sit up without great difficulty; will probably recover.

In the foregoing experiment the test animal received serum treatment on the fifth day after the injection of virus and two days before symptoms of the disease appeared in an incubation of seven days. The disease ran a progressive fatal course despite four injections of serum. The control animal showed evidence of paralysis on the eighth day after infection, with slowly progressing symptoms and with subsequent improvement. This animal is alive at this writing.

Summary.—A summary of the results of the foregoing experiments is as follows:

Five of the six serum-treated animals died, a mortality of 83 per cent. Four of the six control animals died, a mortality of 66 per cent. Of the four animals that received intraspinal serum therapy before the appearance of paralysis, one received one treatment, two received two treatments and one five treatments in the preparalytic stage. In one of these, the incubation period was the same as in the control animal.

TABLE 21.

CHRONOLOGIC TABLE OF PROTOCOLS.

No.	Species.	Generation.	Date of Injection.	Incubation, Days.	Amount of Injection, c.c.	Strength, per Cent.	Site of Injection.	Duration of Disease Days	Character of Paralysis.	Outcome.
1	M. rhesus	I	8/12/16	7	0.5	20	Brain	3	Progressing spinal, beginning in left leg...	Died 8/22/16.
					2	20	Perisciatic			
					5	20	Peritoneum			
10	M. rhesus	II	8/23/16	7	0.5	20	Brain	3	Progressing spinal, beginning in left leg...	Died 9/1/16.
					2	20	Sciatic			
					10	20	Peritoneum			
50	M. rhesus	III	9/ 1/16	4	0.5	10	Brain	3	Slight weakness in left leg.............	Recovered without paralysis.
					2	10	Sciatic			
					10	10	Peritoneum			
51	M. rhesus	III	9/ 1/16	4	0.5	10	Brain	3	General muscular weakness; marantic type	Died 9/7/16.
24	M. rhesus	IV	9/ 9/16	5	0.5	10	Brain	4	Progressing spinal, beginning in left leg; cessation of process; both legs and left arm involved.	Recovered with residuals.
26	M. rhesus	IV	9/21/16	7	0.5 (No. 51)	10	Brain	3	Rapidly progressing spinal, beginning with left leg.	Died 10/10/16.
27	M. rhesus	IV	9/21/16	7	0.5 (No. 51)	10	Brain	3	Rapidly progressing spinal, beginning with left leg.	Died 10/1/16.
36	M. rhesus	V	10/ 2/16	7	0.5	10	Brain	4	Rapidly progressing spinal, beginning with left leg.	Died 10/12/16.
37	M. rhesus	V	10/ 2/16	7	0.5	10	Brain	5	Progressing spinal, beginning with left leg; progressed as far as left arm.	Recovered with residuals.
25	M. rhesus	V	10/ 2/16	13	0.5	10	Brain	20	Slowly progressive spinal, beginning in left leg.	Died 11/3/16.
42	Giant M. rhesus	VI	10/13/16	12	0.5	10	Brain	7	Progressing spinal, left leg.............	Died 10/31/16.
					5	10	Peritoneum			
43	M. rhesus	VI	10/13/16	10	0.5	10	Brain	5	Progressing spinal, left leg.............	Died 10/27/16.
S	M. rhesus	VII	10/31/16	8	0.5	10	Brain	2	Progressing spinal, left leg.............	Died 11/9/16.
					5	10	Peritoneum			
52	M. rhesus	VII	11/ 2/16	7	0.5	10	Brain	5	Progressing spinal, left leg.............	Died 11/13/16.
53	Mangabey	VIII	11/10/16	7	0.5	5	Brain	6	Progressing spinal, beginning in right leg..	Died 11/22/16.
54	Mangebey	VIII	11/10/16	8	0.5	5	Brain	7	Progressing spinal, left leg, and weakness of right leg and left arm.	Appears to be recovering.
23	M. rhesus	VIII	11/15/16	11	0.5	10	Brain	4	Weakness of left and right legs.........	Appears to be recovering.

TABLE

SUMMARY OF EXPERIMENTS ON INTRASPINAL

No.	Species.	Generation.	Virus Injected				Serum Injection Intraspinously		
			Amount, c.c.	Strength, per Cent.	Site.	Date.	Date.	Amount, c.c.	Source, Serum.
50	Rhesus	II	0.5	10	Brain.....				
			2	10	Sciatic.....	9/ 1/16	9/ 6	3.5	102
			10	10	Peritoneum		9/ 7	3.5	102
51	Rhesus Control	II	0.5	10	Brain	9/ 1/16	....	....	....
27	Rhesus	III	0.5	10	Brain.....	9/21/16	9/28	2.5	102
			2	10	Sciatic....		9/29	2.5	102
26	Rhesus Control	III	0.5	10	Brain	9/21/16	....	....	....
36	M. rhesus	IV	0.5	10	Brain	10/ 2/16	10/ 7	3	102
							10/ 8	3	102
							10/ 9	2.5	102
37	Rhesus Control	IV	0.5	10	Brain	10/ 2/16	....	....	....
52	Rhesus	VI	0.5	10	Brain	11/ 2/16	11/ 8	2.5	102
							11/ 9	2.5	102
							11/10	2.5	102
							11/11	2	98
S	Rhesus (large) Control	VI	0.5	10	Brain, intra-	10/31/16	....	....	....
			5	10	peritoneally				
53	Mangabey	VII	0.5	5	Brain	11/10/16	11/15	3	102
							11/16	3	102
							11/18	2.5	98
							11/19	2	98
54	Mangabey Control	VII	0.5	5	Brain	11/10/16	....	....	....
43	Rhesus	V	0.5	10	Brain	10/13/16	10/18	3	102
							10/19	2.5	102
							10/20	3	102
							10/21	2.5	98
							10/22	2	98
							10/23	2	98
							10/24	2.5	102
							10/25	3	102
42	Giant rhesus Control	V	1	10	Brain.....	10/13/16	....	....	....
			5	10	Peritoneum				

22.

TREATMENT WITH UNKNOWN "IMMUNE" SERUM.

			Paralysis.	
Date.	Incubation, Days.	Duration of Disease, Days.	Character, Extent.	Outcome.
9/6	5	2	Slight weakness in left leg..................	Alive and well.
9/5	4	3	General muscular weakness; marantic type....	Died 9/8/16.
9/28	8	3	Rapid progressive paralysis, left leg, right leg, left arm and right arm; resp. failure.	Died 9/30/16.
9/28	8	3	Rapid progressive paralysis, left leg, right leg, left arm, right arm; resp. failure.	Died 9/30/16.
10/9	7	4	Rapid progressive paralysis beginning with left leg; right leg, left arm, right arm; resp. paralysis.	Died 10/12/16.
10/9	7	4 acute	Progressive flaccid paralysis beginning with left leg; right leg, left arm, then regression.	Alive; has diplegia.
11/ 9/16	7	5	Progressive spinal, beginning with left leg; then right leg, left arm, right arm.	Died 11/13/16.
11/ 8/16	9	2	Rapid progressive spinal, beginning with left arm; then right, then resp. failure.	Died 11/9/16.
11/ 7/16	7	6	Progressive spinal paralysis, beginning with right leg; then left leg, left arm, right arm; resp. failure.	Died 11/22/16.
11/18/16	8	8	Progressive spinal paralysis, beginning with left leg; weakness right leg and left arm; slight weakness in right arm.	Alive and improving.
10/23/16	10	5	Progressive flaccid paralysis beginning with left leg, then right leg, then left arm, then right arm; resp. failure.	Died 10/27/16.
10/25/16	12	7	Progressive spinal paralysis, beginning with left leg, then right leg; simultaneously in both arms; resp. failure.	Died 10/31/16.

In the remaining three, the incubation was shorter by one to two days than in the control animals. The duration of the disease in the serum treated animals was somewhat shorter than in the controls, varying from two to six days, whereas in the latter, the acute progressive stage was from three to eight days, indicating a greater rapidity in the spread of the disease in the central nervous system. The number of serum administrations varied from two to eight, depending on the condition of the animal. They were discontinued when the animal exhibited distinct signs of improvement, or when the paralysis had progressed to respiratory difficulty.

DISCUSSION.

Experimental poliomyelitis is several times more fatal a disease than human poliomyelitis, and perhaps it is rather severe on the clinical use of untested "immune" serum to compare it with experimental use of a like serum in monkey poliomyelitis. That, however, seems to be the only method known at the present time of gaining any correct idea of the value of this method of treatment. An effort was made in the foregoing experiments to parallel clinical conditions. Two of the animals were injected with serum on the first day of the appearance of paralysis and four from five to six days after the date of infection, but in the preparalytic stage. The test animals were controlled by six animals that received no serum therapy.

Comparison of the effects of the disease in the serum-treated animals as against the effects observed in the control animals, would incline one to the inference that, at least in the experimental disease, intraspinal injections of serum are not only of no value, but also that there may be in them an element of harm. The mechanism producing the untoward effects has not been demonstrated as yet, but it may consist in the exaggeration of the pathologic process already existing by the introduction into the subdural space of a foreign substance. Marked meningeal symptoms have been observed following the introduction of serum, both in the experimental and human disease. In some instances, the spinal fluids obtained after the injection of serum have shown distinct evidences of accentuated inflammatory reaction, as indicated by increased turbidity, due to increase of cellular elements and increase in the content of albumin and globulin.

That this meningeal reaction may have a deleterious influence on the already existing pathologic process in the cord and brain proper, is not without plausibility.

Careful comparative study of the nervous tissues of the serum treated and the control animals may indicate whether or not this is the true explanation.

PART IV.

Pathologic Report on Forty-three Cases of Acute Poliomyelitis.

H. L. Abramson.

The morbid anatomy of acute poliomyelitis has received careful and painstaking study by a number of European pathologists.

Rissler[1] was the first to describe the anatomic changes in the central nervous system, with a report of three cases, in 1888. The changes described were confirmed by a number of other students of the disease, chief among whom are Wickman[2] with a report of fourteen cases, Harbitz and Scheel[3] with nineteen cases, and Strauss[4] with eight cases.

These anatomic studies are confined chiefly to the nervous elements of the body, comparatively little attention having been paid to the other organs. Flexner, Peabody and Draper[5] studied eleven acute cases with special reference to the changes in the organs other than the central nervous system. They found constant changes in the lymphatic structures throughout the body and called attention to focal degenerative lesions in the liver. The changes in the lymphatic tissues consisted chiefly of hyperplasia of the reticular and endothelial cells.

Though the changes in the central nervous system of acute poliomyelitis as described by Rissler have been confirmed by all subsequent workers in this field, two schools of opinion have developed as to the interpretation of these changes. One group, headed by Rissler, believes that the nerve cell destruction is the primary change and that the cell reaction or inflammatory change has been called forth by the presence of the virus in the affected part. Mönckenberg[6] and Cassierer[7] agree with Rissler, inasmuch as they have studied cases in which there were marked nerve cell destruction with a minimum of interstitial reaction. Wickman,[2] Harbitz and Scheel[3] and others consider that the interstitial process is the primary stage and that the nerve cell degeneration is secondary to the interstitial changes. Wickman has never found ganglion cell destruction without interstitial lesions. On the other hand, he has found interstitial changes without nerve cell degeneration. Harbitz and Scheel state further that the disease is a blood borne infection, the virus lodging first in the meninges, and that it spreads from this site to the nervous tissue proper.

Forssner and Sjövall[8] first called attention to the importance of neurophagocytosis in the production of the histologic picture of this disease.

The epidemic of the summer of 1916 furnished the opportunity for the postmortem study of forty-three patients who had acute poliomyelitis, forty-one of whom died in the acute stage. Thirty-five were cases from the Willard Parker Hospital, and six were in private residences. Material[9] from two were sent to the laboratory for diagnosis. Eight cases other than poliomyelitis came to the necropsy table from the poliomyelitis wards. Four of these proved to be tuberculous meningitis; one lobar pneumonia; one con-

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1. Rissler, John: Nord. Med. Ark., 1888.
2. Wickman, Ivan: Nervous and Mental Disease Monograph, Series 16.
3. Harbitz and Scheel: Deutsch. med. Wchnschr., 1907, p. 48.
4. Strauss, J.: Arch. Pediat., August, 1910.
5. Flexner, Peabody and Draper: Jour. Am. Med. Assn., 1912, 58, 109.
6. Mönckenberg, J.: München, med. Wchnschr., 1903.
7. Cassierer, R.: Neurol. Centralbl., 1898.
8. Forssner and Sjövall: Ztschr. f. klin. Med., 1907.
9. No history obtained of sent-in cases.

genital heart disease; one purulent pericarditis, purulent pleuritis, bronchopneumonia and general pyemia; one, intracranial hemorrhage.

The poliomyelitis cases as to sex, age and duration of illness are divided as follows:

Age	Number
Up to 1 year	8
1 to 2 years	8
2 to 3 years	13
3 to 5 years	2
5 to 10 years	4
10 to 16 years	1
Over 16 years	5

Up to and including 5 years, there was a total of 31, or 75.5 per cent.

Males, 28; females, 13.

Duration of Illness	Number
3 days	10
4 days	5
5 days	6
6 days	5
7 days	5
8 to 11 days	4
Over 12 days	4
Undetermined number of days	2

Thirty-one, or 75.5 per cent. of the patients died within the first week after onset.

The clinical classification as to types is based on the evidence of anatomic lesions. Thus, all cases exhibiting involvement of the lower motor neuron were called spinal cases. These were again divided into two classes: one in which the process begins in the lumbosacral cord and progresses upward involving the arm and respiratory centers, and the other, in which the process begins in the cervical and bulbar regions or in the gray matter from which the cranial nerves have their origin. Those cases exhibiting disturbance of upper motor neuron or other disturbance of the sensorium belong to the cortical type. Those cases showing only marked meningeal symptoms were considered to be of the meningitic variety. They are as follows:

Type of Cases	Number
Ascending spinal	17
Upper spinal	18
Cortical	2
Meningitic	4

In one of the ascending spinal cases the patient survived the poliomyelitis infection and died of lobar pneumonia thirty-four days after the onset of illness. Another patient, belonging to the upper spinal group, died of acute gastro-enteritis twenty-eight days after the onset of the poliomyelitis infection.

The gross changes, when in the brain, were chiefly those of varying grades of congestion of the pial and parenchymatous vessels; of edema of the pia and brain substance. In a few instances, the brain tissue was of softer consistency than normal. One brain, in a man of 27 years, besides intense congestion and edema, presented extreme softening in one hemisphere involving the motor area and a great portion of the parietal lobe, in which lies the sensory area. Clinically, this man presented a hemiplegia and hemianesthesia of the opposite side. The brain tissue was reduced to mushy consistency with multiple hemorrhagic flecks throughout the cut surface. No gross hemorrhage was visible and the spinal fluid was clear, with changes such as one would find in poliomyelitis. The Wassermann was negative. This is the only cortical case of simulating apoplexy that came under observation at the Willard Parker Hospital among a great number of cases of poliomyelitis. The changes in the spinal cord were observed mainly on cut sections through the pons, medulla and upper cervical portion, it being our intention to preserve as much as possible of the material in a clean state for cultures, microscopic study and animal experimentation. The cut sections presented degrees of hyperemia and swelling of the gray matter. The gray "H" bulged above the level of the surrounding white matter and was sharply demarcated from same. In marked cases, the gray matter was simply pink tinged and easily marked off from surrounding white matter. Sections through normal cords fail to show the ready differentiation between gray and white matter which is to be observed in cords from poliomyelitis infection. In some cases the edema involved the white matter as well and appeared to soften the cord as a whole.

TABLE OF GROSS AND MICROSCOPIC CHANGES.

No.	Name.	Sex.	Age in Years.	Duration of Illness, Days.	Type of Case.	Gross Changes.					Microscopic Changes.					Remarks.
						Brain.	Cord.	Mes. Node.	Peyer's Patches.	Spleen.	Brain.	Cord.	Mes. Node.	Peyer's Patches.	Spleen.	
1	R. S.	♀	1 6/12	11	Ascending spinal	++	++	+	?	++	++	++	+	+	+	
2	R. X.	♀	1 1/12	7	Upper spinal	+	+	—	—	—	+	++	—	—	—	
3	G. B.	♀	1 7/12	6	Upper spinal	+	+	+	±	+	+	+++	+	+	+	
4	M. F.	♂	2 6/12	3	Upper spinal	+	—	?	+	—	+	++	+	+	+	
5	C. P.	♂	8/12	6	Ascending spinal	++	?	—	+	+++	+	+	+	+	++	
6	J. N.	♂	2 6/12	3	Upper spinal	++	+	+	±	+	+	++				
7	I. S.	♂	3	4	Upper spinal	++	+++	+	+	++	±	+	—	—	—	
8	M. L.	♀	15 6/12	5	Ascending spinal	+++	?	—	—	—	+	+	..	..	..	Partial necropsy
9	E. C.	♂	7/12	6	Ascending spinal	±	+	+	+	—	+	+	+	—	—	
10	M. D.	♂	2 6/12	7	Upper spinal	+	+	++	+++	—	+	+	+	+	+	
11	I. F.	♂	2	5	Upper spinal	++	++	—	—	—	+	+	..	..	..	Partial necropsy
12	G. W.	♂	21	9	Ascending spinal	+	++	—	—	—	+	++	—	—	—	
13	N. H.	♀	1 11/12	10	Ascending spinal	++	++	+	+	—	+	++	—	—	+	
14	A. L.	♂	2	7	Ascending spinal	++	..	—	?	+++	+	++	—	—	+	
15	A. R.	♂	8	4	Cortical	++	..	++	++	—	..	..	..	..	..	Sections lost
16	A. L.	♀	2 6/12	?	Upper spinal	+	..	—	++	—	+	++	±	±	±	
17	I. N.	♂	8/12	28	Upper spinal	±	..	—	—	—	±	±	—	—	—	Died of acute gastro-enteritis
18	M. K.	♀	6	7	Ascending spinal	+	..	+	?	—	+	+	+	+	+	
19	E. A.	♂	32	5	Ascending spinal	+	+	—	—	+	+	++	—	—	+	
20	H. V.	♂	7/12	? 7	Ascending spinal	+	±	±	+	+	+	+++	±	+	+	Diffuse infiltration predominates
21	S. G.	♂	2 6/12	3	Upper spinal	+	+	+	?	+	+	++	+	+	+	
22	A. K.	♀	4	3	Upper spinal	+	..	+	±	+	+	++	+	+	+	
23	R. C.	♀	2	5	Meningitic	+	..	?	—	+	+	+	—	—	+	

TABLE OF GROSS AND MICROSCOPIC CHANGES.—Continued.

No.	Name.	Sex.	Age in Years.	Duration of Illness, Days.	Type of Case.	Gross Changes.					Microscopic Changes.					Remarks.
						Brain.	Cord.	Mes. Node.	Peyer's Patches.	Spleen.	Brain.	Cord.	Mes. Node.	Peyer's Patches.	Spleen.	
24	R. S.	♀	3	3	Upper spinal	+	..	+	+	+	..	..	..	..	..	Sections lost
25	S. R.	♀	1 5/12	4	Upper spinal	+	+	+	?	+	+	++	—	—	±	
26	J. S.	♀	1 6/12	4	Ascending spinal	++	+	+	+	+	+	++				
27	A. K.	♀	9/12	6	Meningitic	+	..	—	—	—	..	..	..	..	..	Sections lost
28	M. G.	♂	9/12	34	Upper and lower spinal	+	..	—	+	+	+	++	+	+	+	Lobar pneumonia
29	G. C.	♂	1 6/12	4	Upper spinal	+	..	+	?	+	+	++	+	—	+	
30	P. C.	♂	24	6	Ascending spinal	±	..	—	—	+	±	++	—	—	—	
31	S. G.	♂	1 6/12	3	Upper spinal	..	..	+	+	+	+	++	—	—	+	
32	K. V.	♂	3	3	Ascending spinal	+	?	..	..	..	+	+++				
33	B. V.	♂	6/12	3	Upper spinal	+	+	±	—	—	+	+	—	—	—	
34	F. S.	♂	2	?	Upper spinal	+	—	—	±	—	±	+	+	+	+	Moribund on admission
35	E. P.†	♂	2/12	13	Ascending spinal	±	±	—	—	±	±	+	—	—	—	
36	I. K.	♂	27	5	Cortical	++	+	?	?	—	..	..	..	..	..	Sections lost
37	D. M.	♂	5 6/12	5	Upper spinal	+	+	+	+	—	±	+	±	±	±	
38	J. A.†	♂	4	3	Ascending spinal	+	+	—	—	+	±	+	—	—	+	
39	G. B.†	♂	58	22	Meningitic	+	..	..	..	..	+++	+	..	..	..	Partial necropsy
40	A. V.†	♂	2 2/12	3	Upper spinal	+	+	—	±	+	+	+	—	±	+	
41	W. C.‡	♂	?	?	?	..	..	..	..	..	..	++	?	?	?	Cord only was sent to laboratory
42	H. B.‡	♂	?	?	?	..	..	..	..	..	..	++	?	?	?	Cord only was sent to laboratory
43	H.†	♂	9	10	Meningitic	+	+	..	..	..	+	+	..	..	..	Only cord and brain examined

* Explanatory: — = no pathologic change; ± = slight change; + = distinct change; ++ = marked change; +++ = very marked pathologic change.
† Necropsy at home. ‡ Place of necropsy unknown or none had. In all other cases necropsies at Willard Parker Hospital.

The heart and lungs showed no striking changes, except that practically all of the lungs presented acute edema and congestion incident to the paralysis of respiration.

The liver and kidneys showed varying degrees of acute congestion, and in some cases, parenchymatous degeneration.

Particular attention was paid to the lymphatic structures, as some observers contend that lymphoid tissue plays a considerable rôle in the pathologic picture of acute poliomyelitis. The lymphoid structures of the small intestines, the Peyer's patches and solitary follicles, in a number of instances were considerably raised and reddened. There were no indications of ulceration. The mesenteric lymph nodes were enlarged and reddened. The spleen, in many cases, showed marked congestion and more or less prominence of the malpighian bodies.

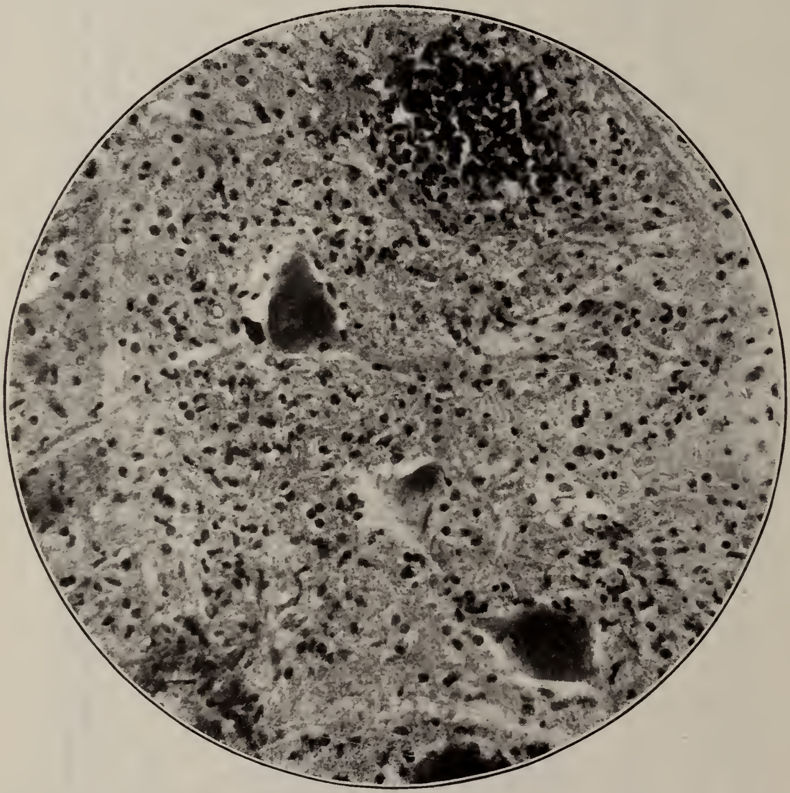

Fig. 1.—Cord showing considerable round cell infiltration. Two of three nerve cells are fairly well preserved. One of these, however, shows early phagocytosis.

Following is a tabulation of gross changes as described in the foregoing:

	Present	Absent	Not Noted or Examined
Brain	35	5	..
Cord	20	5	15
Intestine	16	8	16
Mesenteric nodes	19	10	11
Spleen	18	16	6

A number of the necropsies were granted only for the examination of the brain and cord, and this fact accounts for the incompleteness of the data as to the other organs.

The microscopic pictures of the central nervous system in this study correspond closely with those described by Rissler, Wickman and Harbitz, and Scheel. The disease process involves all the elements that enter into the makeup of the central nervous system.

The pia mater in most cases was affected most severely in that portion which is in direct relation to the most severe process in the subjacent cord. Thus, in the ascending spinal type the most marked changes are to be found in the pia of the lumbosacral region, whereas in the upper spinal type the severest changes will be found in the pia of the upper cord, medulla or pons. However, quite a number of sections presented intense round cell infiltration of the cord proper, whereas the pia of the corresponding level showed little or no cell invasion. These changes consist of congestion of the blood vessels, round cell infiltration of varying degree, both around the blood vessels and also diffusely in the interstitial tissue, and edema, all of which produces a thickening of the membrane. Of the thirty-nine cases, only two failed to show infiltrative changes in the pia of the cord in any of the sections studied. In six, these changes were very slight, consisting mainly of congestion and a slight degree of perivascular infiltration. Two of these were seven and five day upper spinal cases. In the five day case, lesions were found only in the pons and medulla. In one of the remaining cases the inflammatory changes both in the pia and cord proper were slight, but the extent of nerve cell destruction was out of proportion to that of the inflammatory process. In

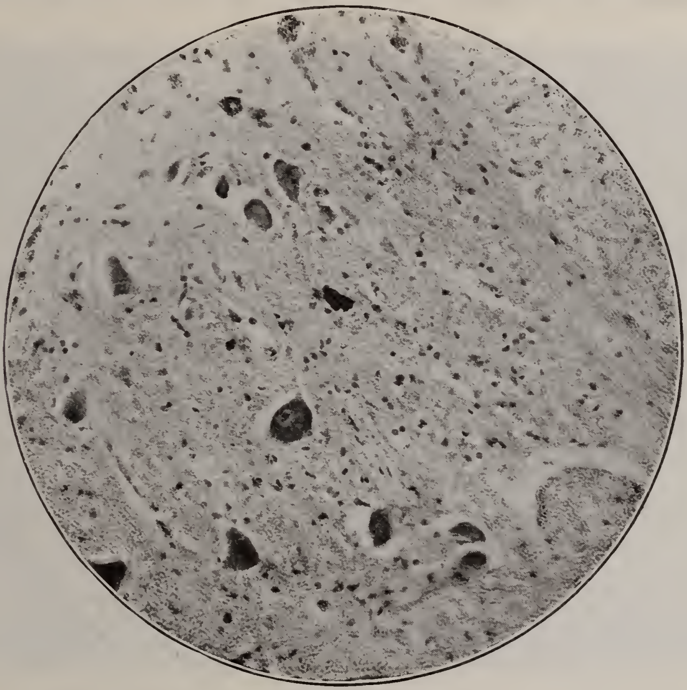

Fig. 2.—Section of cord; marked nerve cell degeneration without round cell infiltration.

another case, while there was only a considerable degree of congestion and edema of the pia without cellular infiltration, the gray matter was densely infiltrated with cells.

The pia of the brain and cord presented changes varying from simple congestion and edema to a marked degree of cellular infiltration and thickening of the membrane.

Spinal Cord.

The spinal cord presented changes of the most severe type usually in that part of its extent that was diseased as indicated clicinally by the flaccid paralysis, usually in the lumbar and cervical enlargements. These changes consist of varying degrees of perivascular infiltration; diffuse interstitial cellular infiltration of the gray matter and occasionally of the white matter; of degeneration and phagocytosis of the ganglion cells, most marked as a rule in the anterior horns, and some degree of edema. Ten of thirty-nine cases examined exhibited hemorrhage in one or both anterior horns. Cords in which hemorrhage occurred presented evidences thereof at all levels in varying degree. The gray matter was particularly affected. Ganglion cells were severely damaged. There was, however, little or no round cell infiltration. There was marked edema. The most marked ruptures occurred in the anterior horns, and in one the horn was almost completely destroyed by the extravasation. Fibrin deposit or other evidence of thrombosis was not noted in any of the vessels.

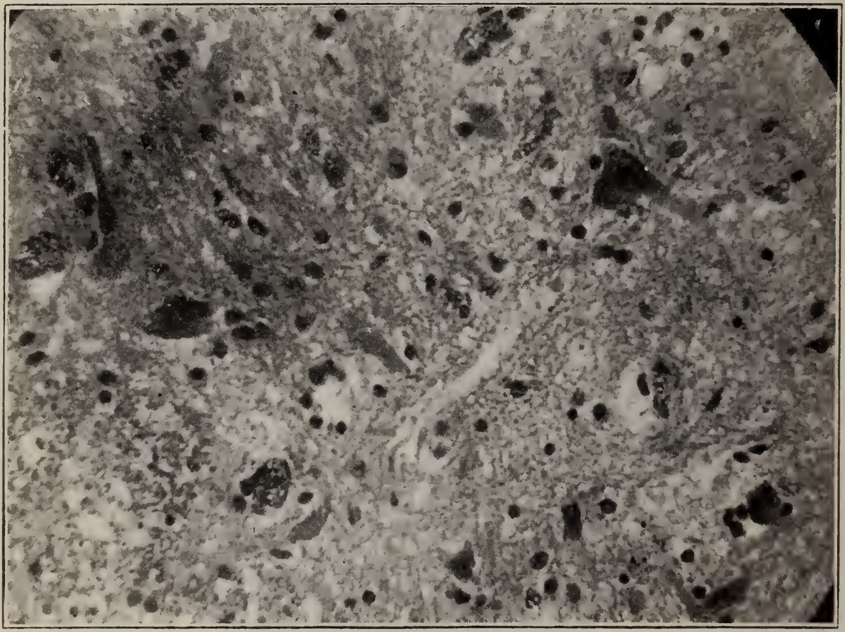

Fig. 3.—Section of cord showing considerable nerve cell degeneration with little or no cell infiltration.

The perivascular infiltration is most marked as a rule about the vessels in the anterior and posterior median fissures, and in the vessels of the tips of the horns. The vessels of the horn proper seldom show a marked degree of perivascular infiltration, whereas the two vessels, one on each side of the central canal, while they exhibit extreme dilatation and congestion, have not presented infiltration in any of the cases studied thus far.

The most intense diffuse infiltration is usually to be found in the anterior horns. In most of the cases studied, however, the whole of the gray matter participated, sometimes more on one side than another. In one case the posterior horn showed the greater cellular infiltration. It was noted, too, in a number of cases that where the diffuse interstitial infiltration was marked, the perivascular change was less marked and vice versa. In a few cases it was noted that at the lower levels of the cord the diffuse infiltration was marked and the perivascular changes not very severe, whereas at the upper levels of the same cord the perivascular infiltration was very severe while there was little or no diffuse interstitial infiltration. This would indicate that the perivascular infiltration precedes the diffuse cellular infiltration and that the latter is derived from the former.

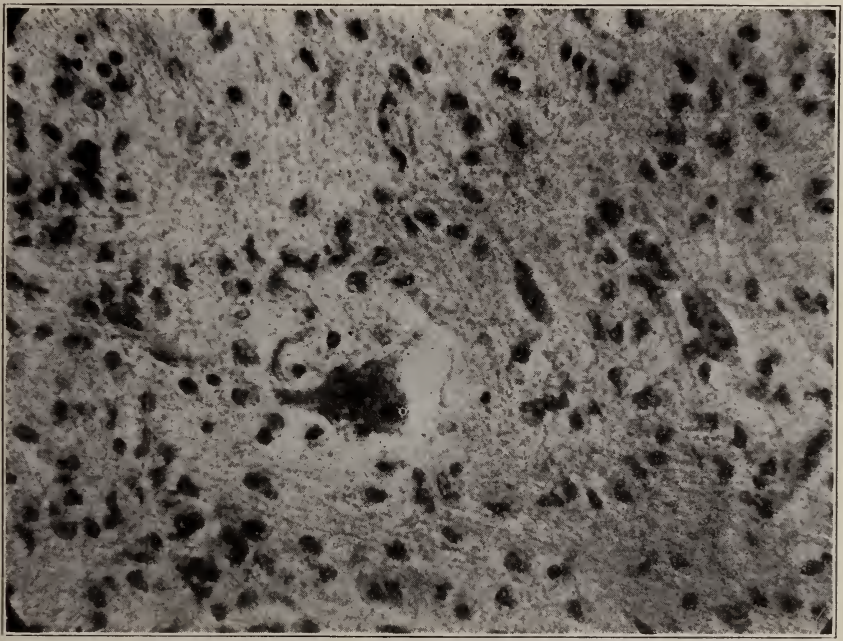

Fig. 4.—Section of cord; invasion of degenerated nerve cell by phagocytes.

The cellular exudate consists largely of mononuclear cells, with a small sprinkling of polymorphonuclear cells. In two of the cases, the polymorphonuclears were the predominating cells. The mononuclears are of two varieties; one, with a round, deep blue, evenly stained nucleus with only a scanty amount of protoplasm around it; the other, with a nucleus which may be round or ovoid, pale staining, reticulated and with a moderate amount of protoplasm around it. This is the polyblast as described by Maximov. It probably represents the first type in an active state. The nuclei of the polyblast assume odd shapes, such as the dumbbell, and the crescent. In some cells, there is a division of the nuclear material forming the polyform cells described by Flexner as being present in the spinal fluid of monkey poliomyelitis. The first type of mononuclear cell predominates in the perivascular infiltrations, whereas in the diffuse interstitial infiltrations the polyblast is the predominating cell type. This peculiar distribution can be readily explained by supposing that the cells in the perivascular infiltrations are in the process of mobilization, whereas those of the interstitial tissues are in the active process of destroying the virus and carrying off destroyed nerve cells. This would account for the change of the mononuclear into the polyblast. The source of these cells is undoubtedly the lymphatic system, which discharges them into the central nervous system directly by the perivascular lymph spaces and indirectly by the blood stream. It is possible that the glia takes part in the acute infiltrative process, but it is not very probable. Certainly the glia cells of the white matter show very little activity, and if they were responsive to the invasion of the virus, it is to be expected that they would proliferate more than is evident from study of the sections. There can be no doubt as to the part the glia plays in the process of repair, but it participates little if any in the acute infiltrative process.

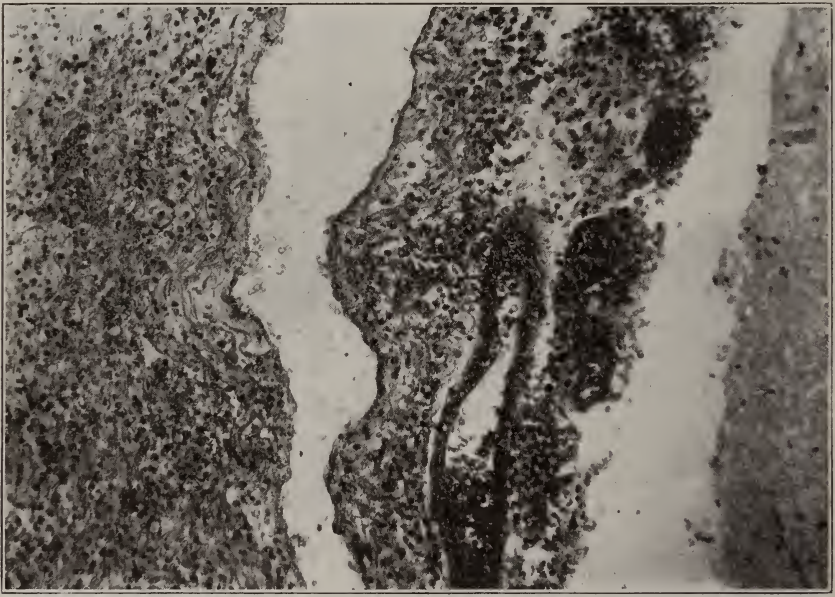

Fig. 5.—Pia from brain showing great thickening due to edema and cellular infiltration. Blood vessels are dilated and engorged.

Infiltration of the tissues with fluid was noted as being present in the gray matter in varying degrees and in not a few instances also in the white matter. The edema appears to make the tissue more spongy and separates the reticular meshwork. As Wickman has pointed out, the recession of edema may have much to do with the rapid disappearance of paralysis that is often observed in this disease.

Destructive effects on the nerve cells were noted in all the cases, those most commonly affected being in the anterior horns. Clarke's column suffered in those cases in which the posterior horns were markedly affected. In most cases, the intensity of the nerve cell destruction varied directly with the degree of interstitial cell infiltration. There were many sections, however, in which the nerve cell destruction was markedly out of proportion to the degree of cellular infiltration. A number presented extreme ganglion cell destruction with practically no interstitial change. On the other hand, there were cases with marked cellular infiltration in which the cells were in fair state of preservation. These are observations that corroborate the findings of Rissler and his followers.

The changes in the nerve cell progress from the loss of the Nissl's granules to complete hyalinization, in which all nuclear material is absent. In those cases in which the cell infiltration is marked, hardly a nerve cell is to be found. Here also are to be seen polyblasts arranged in small groups in positions formerly occupied by nerve cells. This represents complete phagocytosis. Other degenerated nerve cells are to be seen in the process of being invaded by polyblasts. In those cases in which the cellular reaction is not marked and where considerable nerve cell damage exists, there are to be seen numbers of nerve cells in the state of hyaline degeneration. On the other hand, as already stated, where there is a marked cell reaction, there are few nerve cells to be seen. This is an observation which is pertinent to the question of the rôle performed by the interstitial elements in the pathology of this disease.

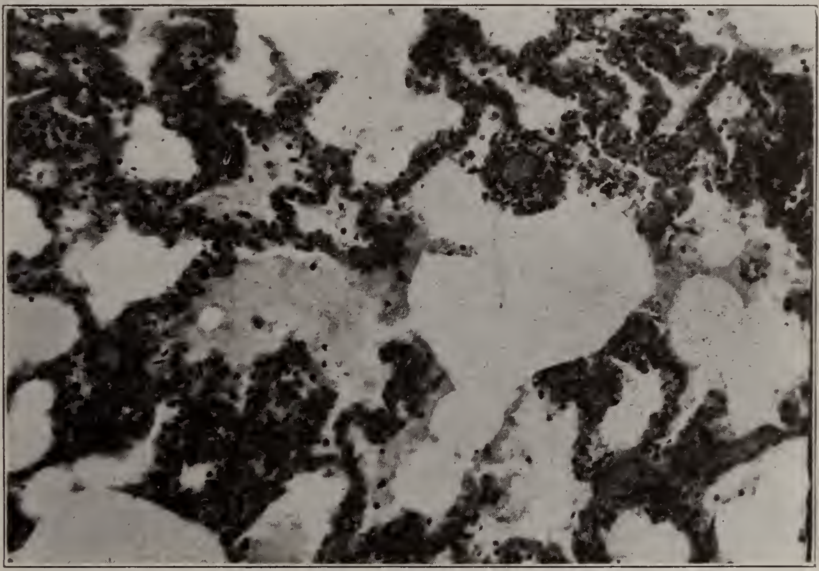

Fig. 6.—Section of lung showing thickened alveolar walls due to infiltration with small round cells.

The pons and medulla exhibited round cell infiltrations and degenerative changes in thirty of the thirty-five cases examined. The remaining five showed marked congestion of the blood vessels. In one case, the pons and medulla presented the only changes found in the central nervous system. The cell infiltrations for the most part were located in between the bundles of fibers, with no tendency to localize in any particular region. Rarely were they found in the region of the nuclei. In nine of the cases there was marked diffuse infiltration. One section presented a large hemorrhage. The nerve cell changes were not as marked as in the cord. Often one group of nerve cells exhibited degenerative changes, whereas an immediately adjacent group would appear quite normal.

The changes in the cerebrum and cerebellum were very mild when compared with those in the cord. The most common change consisted of engorgement of the vessels, the endothelium of which had the appearance of being swollen. In fourteen of thirty-seven cases the brain exhibited very mild cell reactions, consisting in slight degree of perivascular infiltration and, in a few cases, in an accumulation of cells apart from the vessels. The nerve cells exhibited no degenerative changes. The cerebellum exhibited similar changes in five of thirty-three cases examined. These changes were most commonly found below cellular layers in the cerebrum and below the granular and Purkinje cell layers in the cerebellum, in among the fibers radiating from the cortex. It is unfortunate that the sections of the two cortical cases have been lost, as they would undoubtedly have presented interesting changes.

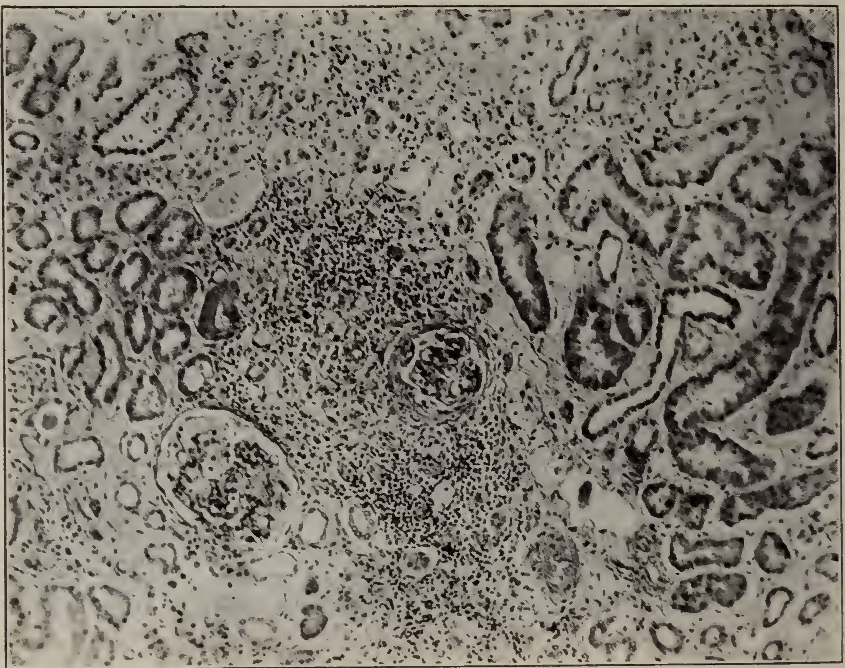

Fig. 7.—Section of kidney showing round cell infiltration and degeneration of glomeruli and tubules.

CHANGES IN OTHER ORGANS

Careful studies were made of the lymphatic structures, particularly the agminated and solitary follicles of the small intestine, the mesenteric lymph nodes and the spleen. The thymus gland was studied in a few cases. Of twenty-nine cases in which the small intestine and mesenteric nodes were examined, sixteen presented distinct changes. In twenty-one of thirty-one cases the spleen presented similar changes. These consisted of a simple congestion of the blood vessels and distinct proliferation of the reticular cells, and marked hyperplasia of the endothelial cells. The germinating centers were increased in number and size and were very active. Moderate numbers of mitotic figures were present. The spleen in a number of instances presented intense congestion which dominated the picture. In no case was there attempt at ulceration of the intestinal mucosa over the lymphatic structures, and the adjacent mucosa failed to present cell infiltration such as one would expect in case of an active local inflammation, as in typhoid fever. In a number of cases, the lymphatic nodules adjacent to the bronchioles in the lungs exhibited changes as above described. The lung capillaries were very often intensely engorged and tortuous. In a number of cases air vesicles presented an accumulation of fluid and desquamated epithelial cells. The majority of the lung sections examined presented a marked thickening of the alveolar walls, due in part to engorgement of the capillaries, but in the main to round cell infiltration. Some of the blood vessels presented a narrow collar of these cells very much like the perivascular changes observed in the cord proper. These changes may be the pathologic basis of the so-called "paralytic râles" observed by Louria.

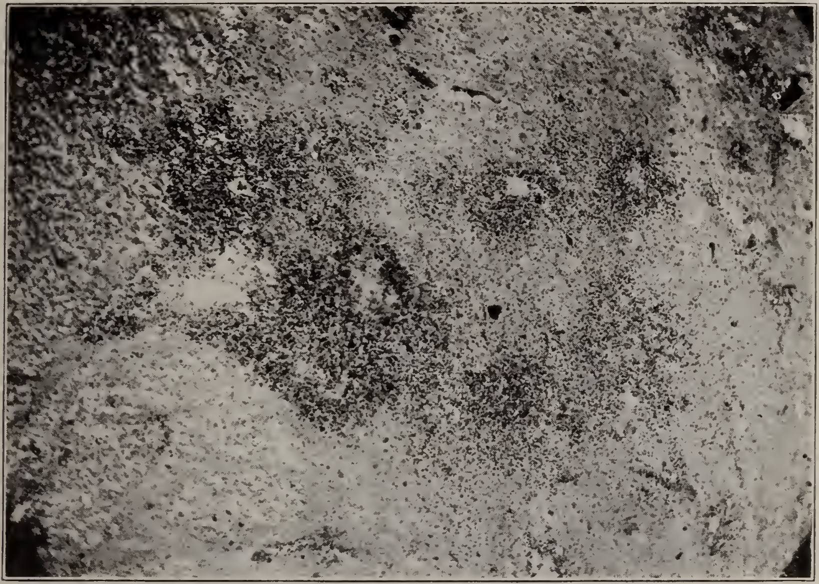

Fig. 8.—Large hemorrhage into cord.

The heart presented congestion of the small vessels, in many of which there was evidence of hyperplasia of the endothelial cells. In some instances there was slight perivascular arrangement of mononuclear cells, such as Wickman had described in the subpericardial fat. These changes were noted only about thin walled vessels in the connective tissue septa between the muscle bundles. The larger, thick walled vessels did not exhibit such changes. The muscle fibers showed no pathologic change.

The liver, in quite a number of instances, exhibited an increase in small round cells in Glisson's capsule and some of the thin walled vessels presented endothelial hyperplasia. The entire vascular system was intensely engorged in many instances. Focal necroses such as described by Flexner, Peabody and Draper, were not noted, though in some cases there was marked hyaline degeneration of liver cells throughout a section and not localized in foci.

The kidneys in practically all instances were intensely engorged throughout both the cortex and medullary portion. In some cases there was to be seen hyperplasia of the endothelium of the malpighian tuft, so that it would appear as a ball of cells. These tufts appeared slightly smaller than adjacent tufts in which this hyperplasia was absent, perhaps due to the weight of the cells which prohibited the dilatation of the capillaries. Only occasionally was there evidence of parenchymatous degeneration. In two cases the kidneys presented focal accumulations of round cells, chiefly in the cortex. There was no evidence of a chronic inflammatory process such as one would encounter in tuberculosis. The masses of round cells were disposed particularly around the glomeruli and produced degenerative changes in these, possibly by pressure. The cells infiltrated the spaces between the tubules, distorting relationship to each other and to the glomerulus. The tubular epithelium presented granular degeneration. Bowman's capsule was swollen and presented hyaline change. The capillaries were engorged. The changes presented all the characters of an acute process, except that the cell element was the mononuclear instead of the polynuclear. Whether these kidney changes are manifestations of the systemic disease is problematical. However, they are suggestive.

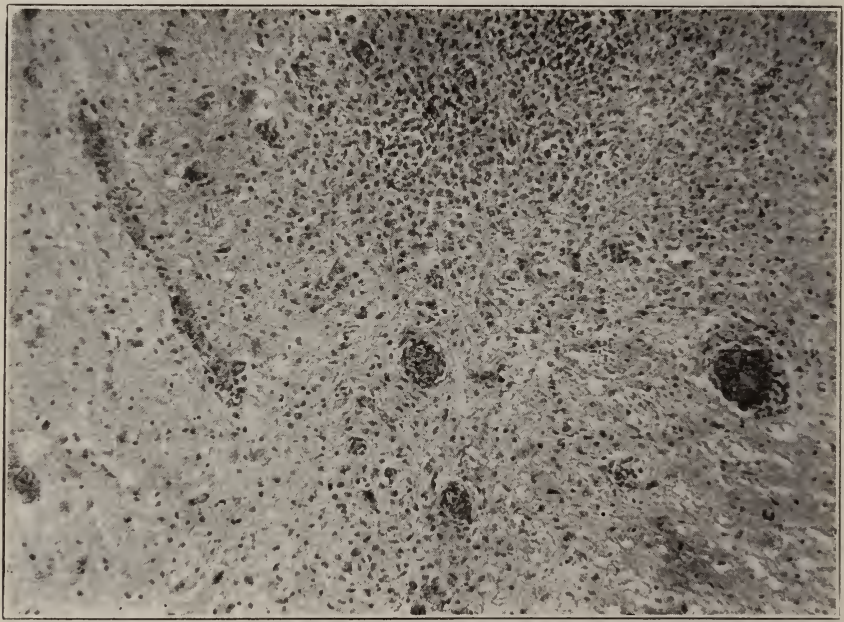

Fig. 9.—Section of cord. Marked diffuse infiltration; very slight perivascular cell infiltration.

The pancreas and suprarenal capsule, aside from congestion and occasionally hyperplasia of the lymphatic elements, presented little that was abnormal.

The question arises, what is the significance of the changes observed in organs other than the central nervous system? The changes in the intestine are not such as to lead one to suspect it as being the portal of entry. The changes in the lymphatic system and endothelial hyperplasia observed in the other organs are undoubtedly a general response of the body cells to an intense local infection situated in the central nervous system. Poliomyelitis is a local infection inasmuch as the chief anatomical lesions are situated in the central nervous system, but this local condition may engender a general response, just as in pneumonia the lung is the focus and the toxemia and the defensive response are general. The changes in the organs other than the central nervous system are not of a destructive character, but rather of a defensive nature.

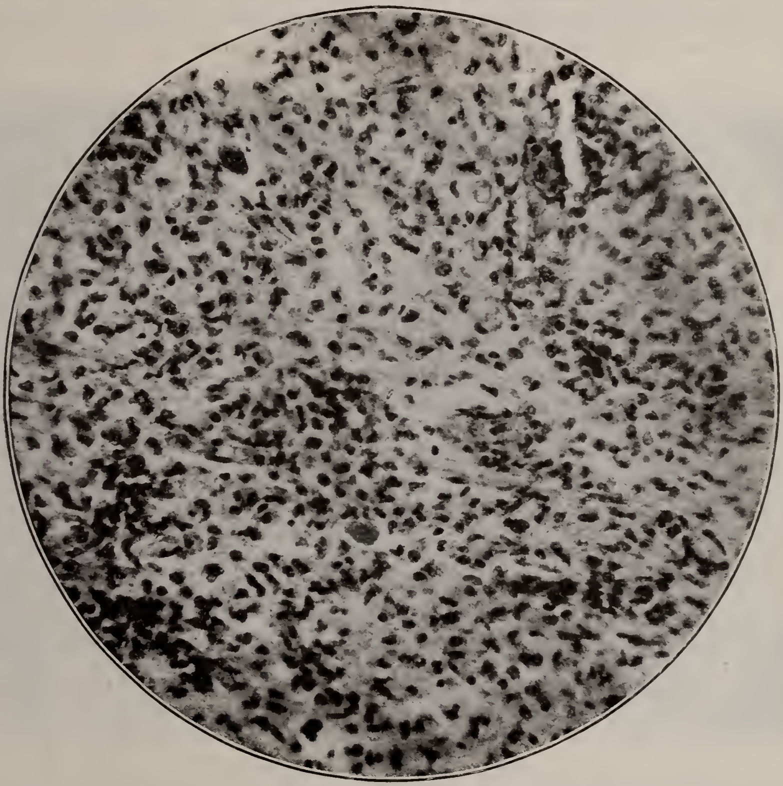

Fig. 10.—Section of cord showing intense diffuse cell infiltration but no perivascular infiltration.

PATHOGENESIS

The question of pathogenesis has divided the workers in this field into two groups. One contends that the damage to the nerve cell is the primary lesion and the evidences of inflammatory changes are secondary; the other, that the inflammatory changes are the direct cause of the changes observed in the nerve cells. Charcot[10] was the first to bring forth the first view. He was supported in this view by Rissler, who was the first to describe the pathologic changes in acute poliomyelitis.

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[10] Charcot and Jaffray: Arch. physiol. norm. et pathol., 1870.

Roger and Damaschino[11] were the first to advocate the primary importance of the interstitial changes. They have received strong support from Wickman and Harbitz and Scheel, and a number of the more recent students of the disease. Wickman differs from Harbitz and Scheel in that he believes that the virus reaches the spinal cord by the way of the perineural lymphatics, whereas the latter authors contend that the path of infection is through the blood stream.

That the virus of poliomyelitis has a specific affinity for the nerve cells of the spinal cord, particularly those of the anterior horn, is indicated by the fact that no matter where the virus is introduced it reaches the spinal cord, whether by the subcutaneous, blood, intestinal, respiratory or intracerebral routes. The behavior of the virus in this respect is analogous to that of rabies. The bulk of experimental evidence thus far adduced strongly indicates that the virus reaches the central nervous system by way of the perineural lymph channels. It has been proved that the rabic virus takes a similar path to reach the central nervous system. The majority of the workers concede that the perineural lymph route is without a doubt the path of infection. Harbitz and Scheel, however, contend that the virus reaches the pia from the blood and spreads from this site to the cord itself along the sheaths of the blood vessels. Experimental work, however, has not supported this idea.

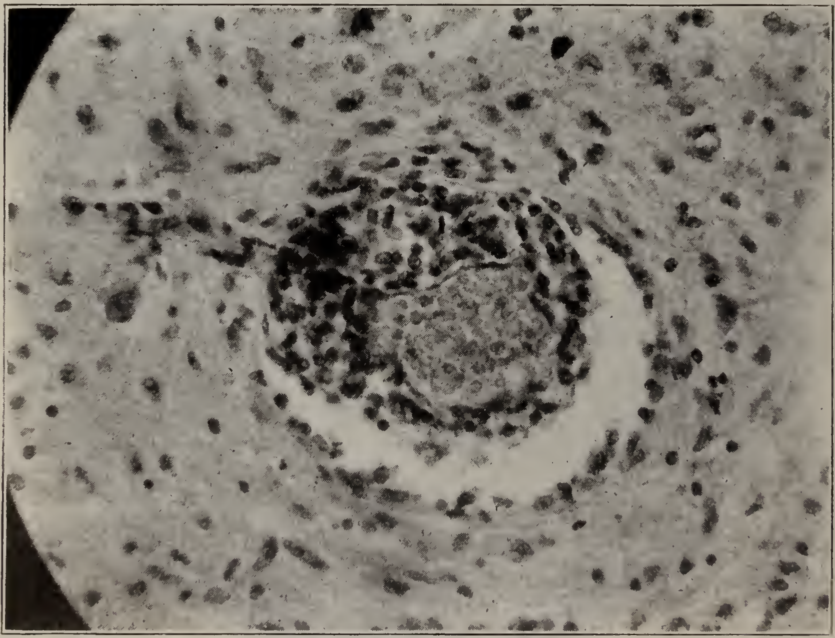

Fig. 11.—Section of cord. Perivascular cell infiltration; very little diffuse infiltration.

It would appear that Wickman's ideas of the pathogenesis are somewhat inconsistent. To grant that the virus reaches the cord by the perineural lymph channels, that the lymph which bathes the nerve cell contains the virus of poliomyelitis, and yet to consider that the nerve cell destruction is brought about by the interstitial phenomena is at variance with what is logical in such circumstances. Furthermore, Rissler, Cassierer and also the present study, present evidence which would indicate that the marked nerve cell destruction can exist with a minimum of inflammatory reaction. Kling, Petterson and Wernstedt[12] have observed changes in monkey poliomyelitis which they described as purely degenerative in character. Because interstitial changes occur in the great majority of cases along with nerve cell changes is no reason for the assumption that the former produces the latter. The fact that there is evidence, even though small in quantity, that nerve cell destruction may be present with a minimum of cell reaction, is sufficient to overbalance the fact that nerve cell destruction is so prevalent in sections showing marked cellular infiltrations. To assume that polyblasts have the power of invading healthy and intact nerve cells and destroying them, is rather far fetched. A more logical assumption would be that the nerve cells being destroyed or damaged are fit subjects for the phagocytic activities of the polyblasts. This, too, would explain why in those sections that present marked cell destruction there is a marked accumulation of polyblasts. Furthermore, it would explain why apparently healthy and intact nerve cells are present in the midst of a densely infiltrated zone without the sign of being subjected to phagocytosis. Those cases in which the interstitial change is so slight, though there is considerable evidence of nerve cell destruction, can be explained by diminished ability to react to the virus, which primarily affects the nerve cells.

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[11] Roger and Damaschino: Rev. de méd., 1881.

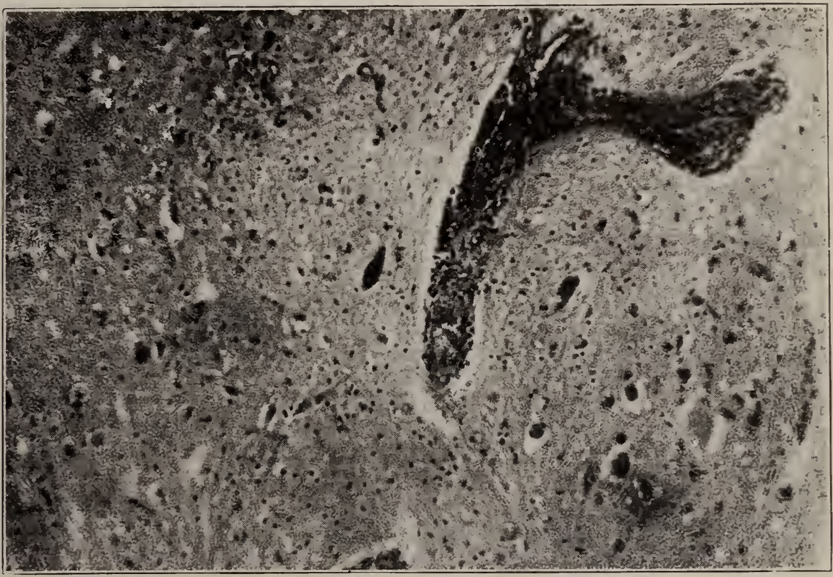

Fig. 12.—Section of cord. Marked perivascular cell infiltration; slight diffuse infiltration.

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[12] Kling, Petterson and Wernstedt: Ztschr. f. Immunitätsf., 12, 316 and 657; 14, 303; 1912, 16, 17.

The pathogenesis of acute poliomyelitis, in the opinion of the writer, is as follows:

The virus has a special affinity for nerve cells, more particularly the anterior horn cells. It reaches the central nervous system by way of the perineural lymph channels. The process spreads in the central nervous system by the lymphatics. The congestion, edema and cellular infiltration, whether present in the pia or in the cord itself, are the cardinal elements of an inflammatory reaction in response to injury produced by the activity of the virus in the cord. The neurophagocytosis is not an active primary destructive process, but rather the exercise of the known normal functions of leukocytes, that of carrying off destroyed material. The congestion and edema, undoubtedly, play a considerable part in the production of transient paralysis through pressure effects.

THE THERAPEUTIC VALUE OF PERTUSSIS VACCINE IN WHOOPING COUGH.*

ANNA I. VON SHOLLY, JULIUS BLUM AND LUELLA SMITH.

This study was made during the later summer of 1916, as part of the investigation by the Research Laboratory of the New York City Health Department on the value of pertussis vaccine in whooping cough.

Because of the infantile paralysis epidemic, car strikes and the expiration, October 1st, of the lease of the building used for clinical purposes, the number of patients available for this study is far less than we could wish. The deductions, however, are suggestive, and show the danger of drawing conclusions too hastily and without making a critical comparison with control cases.

Dr. Luttinger, in his report of May, 1915, pointed out the pitfalls in accepting the statements of patients in the clinic; but in spite of the difficulties he recognized, he gathered from his records that pertussis vaccine lessened the duration of the whoop by 37.5 per cent., as against treatment with drugs. Hoag is enthusiastic about the vaccine. He claims a reduction in the period of the whoop after three to ten injections of the vaccine of from sixteen to nineteen and one-half days. This was in contrast to the duration of from fifty to one hundred and forty-five days, in cases where only one injection was given. Many other physicians, who have received and used the New York Health Department pertussis vaccine, orally and by letter have reported most encouraging results. On the other hand, Drs. A. F. Hess and Matthias Nichol are among those who are skeptical of the marked curative effects of a vaccine. Dr. Hess believes it has some prophylactic value. Before tabulation of our records, we had the impression that pertussis vaccine had had a somewhat favorable influence on the disease, but in scientific work impressions cannot count against the recorded fact. We believe now that the hope for a favorable reaction intensified the memory of those responses which were favorable and impressed these cumulative mental records on our minds more strongly than the unfavorable records. This made the former seem more numerous than they actually were.

Whooping cough is not easily studied. There is not the control of observations as in an illness confining the patient to bed. Like all diseases it varies in degree according to epidemics, season, individual reaction, etc. Members of the same family, ill at the same time, react differently. While one person in a family exhibits a mild and short paroxysmal stage of the disease, another under apparently the same conditions suffers a severe and alarming attack. The other members of the family may escape entirely. It is unsafe, therefore, to draw comparisons between those treated with vaccine and those not treated with vaccine in a single family. Most of our impressions are probably obtained thus.

The only statistics which can be of value are those culled from a large number of cases carefully and critically observed, so that the extreme variations of the disease itself and the errors which must creep in when one has to depend on the word of parent or guardian for details of the progress of the disease are leveled down and reduced to a minimum.

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*Published in Jour. Am. Med. Ass'n., 1917, 68, p.

The personal equation of the examiner, too, must be reckoned with. When results are not striking and sudden (as they are not with pertussis vaccine), it must make a difference whether the interpreter of the symptoms is an optimist or a pessimist.

As Dr. Luttinger has pointed out, the ideal place to study the disease is the hospital, where the children may be controlled and watched by trained observers. Unfortunately, the children brought to the hospital with pertussis, or those who contract it there, do not represent the average. The hospital patients more commonly have advanced cases with complications, or cases so severe that the parents are alarmed. Children with early cases contracted in the hospital usually leave when they have recovered from their primary trouble. On the whole, under present conditions, these cases are not favorable for testing the vaccine.

A pertussis créche in which children could be segregated during the day while the parents are at work, would afford a fair opportunity of study; but, as far as we know there is none. Of course, in such a créche, the child could not be observed at night. It is at this time of the twenty-four hours that the mothers, according to Dr. Luttinger, report favorable results from the vaccine. Unless such a créche could be located in a whooping cough neighborhood, its value, from the sanitarian's point of view, would be minimized by the danger of conveying infection during transportation of the child to it.

In New York at the present time, the clinic is therefore the best suited but not altogether satisfactory place for trying out the effects of the vaccine. Leaving out the question of the danger of transmitting contagion during the journey to and from the clinic and in the waiting room, we cannot get away from the fact that we are obliged to rely for our facts on the untrained, if not ignorant, unintelligent or mendacious parents or guardians. As often as not, they try to answer to please the questioner; either they exaggerate symptoms, hoping for better treatment or they lessen symptoms to get a discharge for the child to go to the country or to return to school, etc.

Unless the child coughs in the clinic or presents some corroborative suggestive symptoms, such as subconjunctival hemorrhage, ulcer of the frenum, or puffy eyelids, how can one be sure that the child has whooping cough. It is not unusual for a young mother to say after one or more visits, "My child does not cough like those I have heard. I do not believe he has whooping cough after all." During last summer, according to subsequent confessions at least, some mothers brought their children for injections "Because they thought 'the needle' would prevent infantile paralysis." The Italians, particularly, feel that the "syringa" is wonderful treatment for anything that happens to ail one. "It makes weak children strong."

In other instances the cases suitable for treatment are cut down by the fact that the parent is apt to wait until the height of the paroxysmal stage before she brings the child for treatment. A vaccine that "cures" when begun at the end of the second or during the third week must be accepted with a grain of salt. One would expect spontaneous recession at this stage. Some observers report giving the vaccine every other day until they get a response—for six to ten or more injections. This would cover a period of two, three or more weeks and, if the injections were begun after the paroxysmal cough or whoop had declared itself, would carry them over into what would ordinarily be the declining stage of the disease.

Because of these difficulties (and others) we were obliged to throw out many of our records in collating our figures. Over 1,000 patients were seen by us from July 10 to September 30, 1916. About 500 records were rejected because the patients presented themselves after the second week of the disease, or because the records were too incomplete; 93, because of questionable whooping cough; 49, because the information was unreliable; 126, because patients had either moved or given a wrong address so that their subsequent history after the closing of the clinic could not be obtained; 6, because they were not at home on several visits of inquiry; 3, because of questionable exposure to whooping cough, and 1, because the original record card was lost.

TABLE 1.

FAMILY IMMUNITY.

CHILDREN IN 67 FAMILIES EXPOSED TO WHOOPING COUGH.

Immune Children.	Number Children in Family.								Total.	
	2	3	4	5	6	7	8	9		
Number non-vaccinated; first exposure in family	11	26	38	44	18	8	8	1	154	
Number inoculated with influenza vaccine	5	5	3	2	3	...	...	1	19	
Number inoculated with pertussis vaccine	6	10	7	3	4	...	...	...	30	203
Susceptible Children.										
Number in partially immune families who contracted whooping cough, *first* exposure in family	...	...	...	...	...	...	...	...	112	
Number in partially immune families who contracted whooping cough, *second* exposure in family	...	2	1	...	...	...	...	...	3	115
										318

For purposes of comparison we inoculated alternate patients with different vaccines, one being made from the pertussis bacillus; and the other from the influenza bacillus. Only patients presenting themselves before the third week of the paroxysmal stage or the third week of the whoop were injected. Injections were intramuscular. Other patients were treated with terpin hydrate by mouth. A few patients were given intramuscular injections of a highly diluted milk solution. This was done in order to contrast the course of the disease in these patients and in those treated with bacterial vaccines.

Patients who returned often enough to the clinic were generally given three to four injections; one injection every other day, and a fourth from five to seven days after the third. The last mentioned injection was given only when the paroxysmal stage was progressing unfavorably.

The intelligence of the guardian or parent who reported the symptoms was noted in a record, careful cross examinations were made to rule out false reports as far as possible, and note was taken of every child actually heard with a typical paroxysmal cough in the clinic. To complete the records in every case in which the patient failed to return to the clinic, home visits were made until the patient was discharged.

In recording the remarks on the charts, the investigators were not biased by a knowledge of the vaccines employed, as they did not know which was which until the study was finished. This secret was safeguarded by the maker of the vaccine.

We wish to remind the reader that we realize that our numbers are much too small to be of full scientific value. Chiefly for the interest of comparison we quote our results on 274 cases.

Grouping together, as did Dr. Luttinger and Dr. Hoag, all our patients treated with pertussis vaccine (136), we find that the whoop averaged thirty-two days. In those treated with the influenza vaccine (94), the whoop averaged thirty-five days. In the controls treated with highly diluted milk injections or terpin hydrate medication (34), the whoop averaged twenty-six days. If we were to make our deductions of the comparative value of the vaccines in these rather few cases, from this first arrangement of averages, we should have to infer that there was not much choice between pertussis and influenza vaccine, and that terpin hydrate by mouth or dilute milk injections were superior in curative effect to either.

It would seem fairer to draw our conclusions from a different arrangement of our figures. In this grouping division was made into those cases which were mild in intensity before vaccine treatment was begun and those that showed a moderate or severe onset before treatment. These again were grouped according to the stage of disease at which treatment began and the apparent effect of treatment upon the cough, the whoop and the attacks of vomiting respectively.

After weeding out the questionable, unreliable and incomplete records, we divided the 274 cases as described above. We judged the intensity of the paroxysms according to the degree of asphyxia exhibited by the color of the face and lips. Many cases can be vouched for as true cases since the patients were seen and heard to whoop in the clinic. In our grouping of the rest we were guided by cross-examination, estimated according to the apparent intelligence of the mother or guardian.

Mild Cases.

The average duration of the *cough* was from 7 to 8 weeks under both pertussis and influenza vaccines, except for those cases in which treatment was begun during the second week of the whoop. In these the cough runs about a week longer under influenza than under pertussis vaccine.

The average duration of the *whoop* in these cases was as follows:—

In cases in which injections were begun *before* the whoop appeared the average duration of the whoop was about two weeks less under pertussis than under influenza vaccine.

In cases first treated during the *first* week of the whoop the period of its duration was the same with both pertussis and influenza vaccines.

Cases in which treatment was begun during the *second* week of the whoop showed four weeks less duration of the whoop under pertussis than under influenza vaccine.

In control cases *not injected* the whoop lasted only two to three weeks.

Vomiting occurred during periods of about the same duration in patients treated with either pertussis or influenza vaccine if the treatment was begun before the whoop developed.

When treatment was begun during the *first* week of the whoop the period during which vomiting occurred was shorter under pertussis than under influenza vaccine.

When treatment was begun during the *second* week of the whoop the period of vomiting attacks was less under influenza than under pertussis vaccine.

In the *non-vaccinated control* cases the results as to vomiting were the same as under pertussis vaccine begun during the second week of the whoop.

In this series of cases pertussis vaccine seems to have the advantage over influenza vaccine and the unvaccinated controls have somewhat the advantage over both.

Moderate and Severe Cases.

The average duration of the *cough* when injections were begun *before* the whoop developed averaged ten weeks under pertussis vaccine and two to three weeks shorter under influenza vaccine.

When treatment was begun during the *first* week of the whoop the duration of the cough averaged the same under both vaccines.

When treatment began during the *second* week of the whoop the cough ran about a week longer under influenza than under pertussis vaccine.

In *non-vaccinated* controls the *cough* continued on an average three weeks longer than in the average cases under either vaccine.

The average duration of the *whoop* was from five to six weeks under pertussis vaccine without regard to the period at which treatment was begun. Under influenza vaccine the period of the whoop was one week longer than this when treatment was begun *before* the development of the whoop or during the *first* week after its devolpment.

In the *control* cases the period of the whoop was still a week longer than under the last mentioned conditions.

The *vomiting* attacks occurred for a longer period under pertussis than under influenza vaccine when treatment was begun before the whoop developed or during the first week. When treatment was begun in the *second* week of the whoop vomiting occurred for about the same period under both vaccines.

In the *control* cases *vomiting* attacks continued for about two weeks longer than in cases treated with either vaccine.

On the whole in these cases influenza vaccine seems to average more points in its favor than pertussis vaccine in relieving symptoms.

Before going further, we wish to repeat that we feel our numbers are too few to do more than demonstrate anew that deductions drawn from small numbers are apt to be faulty.

In 274 cases results were tabulated also according to the mother's or guardian's opinions of the course of the disease. In this list the percentage of children reported distinctly improved immediately after the third injection or third visit is almost the same for cases under either pertussis or influenza vaccine, with 0.7 per cent. in favor of pertussis vaccine. The slight difference here is without significance, and in connection with the use of either vaccine we cannot rule out the possible favorable effect of a non-specific protein reaction.

Among the patients reported slightly *better* after the third injection influenza vaccine seemed to be 8.8 per cent. more potent than pertussis vaccine.

TABLE 2.

PROGRESS OF THE DISEASE AFTER THREE INJECTIONS OR VISITS ACCORDING TO MOTHER'S OR GUARDIAN'S COMMENTS IN 274 CASES.

Treated with	Total No. Cases.	Improved.		Slight Improvement.		No Change.		Worse.	
		No.	%	No.	%	No.	%	No.	%
Pertussis vaccine.........	145	39	26.7	35	23.9	32	21.9	40	27.4
Influenza vaccine.........	113	31	27.4	37	32.7	15	13.2	30	26.5
Terpin hydrate medication or dilute milk injections.	15	5	33.3	2	13.2	4	26.6	4	26.6
Grand Total.......	274	..	....	..	....	..	....	..	....

Judged by those patients reported *stationary* influenza vaccine shows 8.7 per cent. better results than pertussis vaccine.

Judged by those reported *worse* the results given by the two vaccines show a difference of 0.9 per cent., again in favor of influenza vaccine.

Needless to say, the mothers did not know that there were two vaccines used. Their statements were made notwithstanding the fact that the influenza vaccine was more apt to give both a local and febrile reaction than the pertussis vaccine.

Of the non-specifically treated controls (terpin hydrate medication or dilute milk injections), 5.9 per cent. more children were said to be improved after the third visit than among those treated with influenza vaccine; 10.6 per cent. fewer were slightly improved than of those treated with the pertussis vaccine, and 4.9 per cent. more remained stationary. Practically the same number as under pertussis or influenza vaccine, about one-fourth of the cases, progressed unfavorably.

Contrary to our impressions before the results were charted the mother's reports do not make out a case for pertussis vaccine. In the mild cases injected with water made slightly turbid with milk, and thought at the time to be a vaccine by both doctor and parent of the child, the trouble terminated in less time than in cases treated with either pertussis or influenza vaccine. These results were observed in patients injected before the whoop or paroxysmal cough developed or during the first week of the whoop.

Of the twenty-seven patients receiving only two injections of vaccine, if either vaccine had any effect at all, influenza vaccine in some groups did as well as the pertussis vaccine, and often better.

Of the twenty-seven patients receiving only one injection the same may be said.

PROPHYLACTIC VALUE OF THE VACCINES.

Family Immunity.

Of the children exposed to whooping cough in their immediate families, nineteen received three prophylactic injections of influenza vaccine and thirty received three prophylactic injections of pertussis vaccine. Some of these children had a mild bronchial cough at the time of inoculation. These coughs ran a variable time but no case developed into a characteristic pertussis. These children were observed and visited in their homes for several months; none came down with pertussis. One child who had received one injection of pertussis vaccine contracted whooping cough two months afterward. Hoag reports seventeen cases in which persons injected prophylactically did not contract the disease. Luttinger gives a similar report. Hess reports that of 244 exposed patients (in an institution) inoculated prophylactically twenty or 8.2 per cent. developed whooping cough.

From this it might be concluded that both pertussis and influenza vaccine protect against whooping cough. On the other hand we can show also by our figures that in families which showed partial immunity, children not vaccinated against whooping cough did not contract the disease. Of 269 unvaccinated children in such families 155 (58%) escaped whooping cough. Altogether, of 700 exposed children in 243 of our families, 174 or 24.8 per cent., escaped the disease. Of these 700 children we have a record of three children who came down with pertussis on the second exposure in their family.

In nine families totaling forty-seven children, eighteen children who had not had the disease escaped a second exposure in the family; and in one family of five children, one child escaped a third exposure in the family.

In 166 families of 381 children, all of the children either had had whooping cough or were suffering from it at the time of their attendance at the clinic.

Of 243 families, the children of which were exposed to whooping cough, seventy-seven families (31.6 per cent.) exhibited an immunity in some of the children.

In Dr. Luttinger's series of ninety-two families, twenty-seven (29.3 per cent.) showed partial immunity.

In the light of these natural immunes, can we be sure that pertussis vaccine conferred immunity, especially since, according to our statistics, influenza vaccine apparently can do the same?

There were ten deaths in our series; five of the patients had received pertussis vaccine, four had received influenza vaccine, and one had been treated with drugs. Three of those receiving pertussis vaccine had rereceived three or four injections. All the injections were given before the end of the second week.

SUMMARY.

Since whooping cough vaccine statstics under present conditions must be based chiefly on parents' reports, one must use careful judgment in accepting what they say.

Statistics, to be of any value, must be drawn from several thousand cases, as shown by the variable results obtained with the different groupings of our figures.

One must beware of "impressions."

Our figures show that in the first grouping the none-specific influenza vaccine differs very little from pertussis vaccine in influencing the duration of the paroxysmal stages of the disease. In cases grouped according to degree of intensity of disease and stage at which treatment began, judged by the record of the mild cases pertussis vaccine seems to have the advantage over influenza vaccine, and the unvaccinated controls have somewhat the advantage over both. Judged by the record of the moderate and severe cases grouped as above, on the whole influenza vacccine seems to average more points in its favor than pertussis vaccine in relieving symptoms.

Of all the cases, the shortest course was run in our non-vaccinated controls and those receiving inert, milk-colored water.

None of our patients inoculated for prophylaxis with either influenza or pertussis vaccine contracted whooping cough.

A partial immunity was exhibited by 31.6 per cent of our families.

Fifty-eight per cent. of the unvaccinated children in these partially immune families escaped after exposure in the family.

Of 700 children exposed in their families, 24,8 per cent. escaped whooping cough.

Conclusions.

More observations and more critical observations must be made with controls for comparison before the case can be considered made out for the curative and prophylactic value of a specific pertussis vaccine.

THE VALUE OF VENTRICULAR PUNCTURE FOR THE EARLY DIAGNOSIS AND SERUM TREATMENT OF POSTERIOR BASILAR MENINGITIS.*

Abraham Zingher.

The results obtained about a year ago in the treatment of two babies suffering from posterior basilar meningitis who were admitted to the Willard Parker Hospital are of sufficient interest to justify a short discussion of certain features of the disease and a detailed presentation of the clinical and laboratory reports of the cases. In the literature several late cases of posterior basilar meningitis are reported who recovered after ventricular punctures and injections of antimeningitis serum. No particular attention seems, however, to have been drawn in any of the communications to the importance of an early diagnosis before the progressive character of the symptoms had established the diagnosis beyond a doubt, at which time more or less damage to the brain tissue had already taken place.

The pathological anatomy of posterior basilar meningitis shows that the disease is characterized by a yellow fibrinopurulent exudate which fills up the cisterna magna, spreads downward for a variable distance along the posterior aspect of the cord and forward along the base of the brain as far as the interpeduncular space, the optic chiasm and the tips of the temporosphenoidal lobes.

The early symptoms of the disease in infants below six months of age are not very definite and the diagnosis is often difficult to establish. The only symptoms present may be fine tremors of the extremities, temperature 100° to 104° F., and, of special importance, a slight bulging of the anterior fontanelle. The bulging of the fontanelle in an infant who is quiet and breathing regularly should arouse suspicion and lead to a diagnostic lumbar puncture, especially if one or more of the other symptoms are also present. In cases of posterior basilar meningitis in which there is generally an exudate covering and closing the foramina of Magendie and Luschka, the results of lumbar puncture may be significant in one of several ways: The lumbar puncture may yield (*a*) a dry tap, (*b*) a few drops of clear sterile spinal fluid or (*c*) 1 to 2 c.c. of purulent fluid. This pus represents some of the material which had formed and settled down in the lower part of the spinal canal before the openings between the fourth ventricle and subarachnoid space in the spinal cord became closed by inflammatory exudate. The diagnosis is readily established in the last group of cases by examining the purulent spinal fluid. Very little of this fluid can be withdrawn, however, and little if any of the antimeningitis serum injected. The indications then become very definite for a ventricular puncture, the removal of a larger amount of fluid and the injection of a suitable amount of serum.

The diagnosis remains in doubt, however, in the group of cases that show clinically a slight bulging anterior fontanelle, but give a dry tap or a few drops of sterile spinal fluid on lumbar puncture. We might hesitate before proceeding to the somewhat more radical procedure of a ventricular puncture. It would be a mistake, however, to wait for the development of the more marked symptoms of a progressive meningitis, such as opisthotonos, retraction of the head and internal hydrocephalus. These symptoms represent more extensive and often irreparable damage to the brain tissue. In the presence of a bulging fontanelle a dry tap in the hands of one who is expert in lumbar punctures, especially if it also follows a second or a third attempt, should be a clear indication for making a ventricular puncture to establish the necessary diagnosis. A true dry tap can almost always be established by leaving the lumbar puncture needle in place, inserting a second needle into the next space higher up (between the third and fourth lumbar vertebræ) and, after waiting to see if any spinal fluid will escape, allowing some sterile saline to flow into one of these needles. The saline solution will escape through the other needle, showing that both needles have been properly inserted into the subarachnoid space of the spinal canal.

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* Published in Am. Jour. Med. Sci., 1919, 157, p. 58.

If such a case has not been properly treated in the early stages of the disease by ventricular punctures and injections of serum a group of symptoms will gradually develop in the course of the next ten to fourteen days, which are very characteristic but indicate that more or less damage has already taken place. There is an increasing rigidity of the neck, retraction of the head and a moderate or marked opisthotonos. The extremities become extended and spastic, the hands pronated and clenched and the legs adducted and at times crossed. In other cases there may be flexion of the limbs at the various joints. The development of hydrocephalus is indicated by tense and bulging open fontanelles. Dilated pupils, strabismus and sometimes blindness are other symptoms that develop in the later stages. Fever and projectile vomiting are present, and finally death takes place, with progressive emaciation, which is a marked feature of the disease. If the infant should recover from the acute stage of the illness it will, as a rule, develop a permanent hydrocephalus, associated with an impairment of one or more of the sense organs.

In adults cases are occasionally seen that require trephining, ventricular puncture and injection of serum. These patients begin with an ordinary attack of cerebrospinal meningitis and are treated by lumbar punctures daily for possibly a week or ten days. The spinal fluid may become sterile after the second or third lumbar puncture, but it continues more or less turbid. The clinical symptoms persist and become aggravated. If the patient is conscious he complains of intense headache. There is frequently delirium. The pupils are contracted and the fundi show congested or œdematous disks. In such a case the foramina of Magendie and Luschka have probably become closed by the plastic exudate over the base of the brain while the space between the spinal cord and the foramen magnum has remained clear. This results in a retention of ventricular fluid containing meningococci and a dilatation of the ventricles of the brain, while the cerebrospinal fluid produced in the subarachnoid space passes freely down into the spinal canal and appears by lumbar puncture. The antimeningitis serum injected by lumbar puncture can only reach and sterilize the subarachnoid space of the cerebrospinal axis. To reach the ventricles these patients have to be trephined, the lateral ventricles punctured and the serum injected. The puncture and injection of the serum will have to be repeated several times.

In another group of adult cases the patients have gotten over the most acute symptoms and the spinal fluid has begun to clear up distinctly, when there suddenly begins a rapid aggravation of symptoms and the patients complain of intense headache. The eyegrounds show congested or even edematous disks. The spinal fluid is sterile in culture but slightly turbid. If a ventricular puncture is made the fluid is found distinctly less turbid than the spinal fluid and also sterile in culture. In these cases the acute bacteriological infection has stopped both in the ventricles and in the subarachnoid space of the cerebrospinal axis, but the exudate at the base has gradually closed the foramina of exit from the fourth ventricle and the ventricles in the brain are becoming acutely dilated. Repeated ventricular punctures without the injection of serum are indicated in the treatment of these cases. A needle is inserted, according to the method of Kocher, through a small trephine opening 2.5 cm. from the median line and 3 cm. in front of the coronal suture. The needle is passed inward and slightly backward for a distance of about 4 or 5 cm., when the lateral ventricle will be reached.

The differential diagnosis in infants must take into consideration the following diseases:

(*a*) Tubercular meningitis and the different forms of septic meningitis.
(*b*) Meningismus.
(*c*) Pneumonia.
(*d*) Poliomyelitis and polioencephalitis.
(*e*) Meningeal hemorrhage.

Diagnosis. The diagnosis is established by lumbar puncture and the examination of the spinal fluid. If there is a dry tap and the patient shows a bulging fontanelle a ventricular puncture is indicated. In adults the persistence or aggravation of the clinical symptoms and a cloudy but sterile spinal fluid indicate a ventricular puncture.

Prognosis. The prognosis depends to a great extent upon the stage of the disease in which the diagnosis is established and the serum injected into the ventricles. One of the two cases reported in this communication was treated for three weeks with small injections of antimeningitis serum into the spinal canal. The ventricular fluid obtained from the infant at the end of this time showed a great many meningococci, indicating that the spinal injections of the serum had had very little if any effect on the course of the disease. Three ventricular injections of antimeningitis serum, given at intervals of twenty-four hours, produced a sterile fluid, but the disease had progressed too far and the patient finally died, with marked emaciation. The second patient was treated in the early stages of the disease and recovered completely. The prognosis would seem to depend therefore, to a considerable extent, upon the time when the diagnosis is made and proper treatment instituted.

Treatment. The treatment consists of puncture of the lateral ventricles, made daily or every other day, withdrawal of the fluid and injection of a suitable amount of antimeningitis serum. In antimeningitis serum we have an excellent specific remedy for meningococus meningitis; it must, however, be injected in the early stages of the disease and into those places where it will be most active if it is to be fully efficient. The interval between each puncture and the total number of punctures will depend upon the clinical course and the examination of the ventricular fluid. From four to six punctures and injections of serum may be required. The amount of fluid withdrawn from the ventricles varies from 20 to 50 c.c. and the amount of serum injected from 15 to 35 c.c. The treatments should not be discontinued as soon as the ventricular fluid is found sterile on culture, but one or two additional serum injections should be given. Cases of meningococcus meningitis are occasionally observed that are not treated sufficiently with serum and subsequently show relapses, as indicated by an increased temperature and reappearance of meningococci in the spinal fluid, even after negative cultures for several days.

The technic of ventricular puncture is simple but requires certain precautions. The infant should be wrapped in a sheet and placed in the dorsal position, with the head brought near the edge of the table. The area over and around the anterior fontanelle is shaved, if necessary, and sterilized with tincture of iodin. One assistant holds the head of the infant firmly between his hands and another one secures its body. The operator sits at the head of the table. The lumbar puncture needle used in this operation should preferably be not larger than a No. 18 gauge, three inches long and should have a short bevelled point (George Tiemann & Co.). The needle is introduced through the anterior fontanelle on a horizontal line connecting the two lateral angles, 1 cm. from the middle line, in order to avoid injuring the longitudinal sinus. It is passed to a depth of from 2 to 5 cm., when the lateral ventricle will be readily reached. The direction of the needle from its insertion through the scalp is almost perpendicular but slightly outward and forward. The needle is held very gently and supported, rather than firmly fixed, between the fingers. The plunger in the needle is removed and the ventricular fluid allowed to escape into a test tube, which is held by an assistant. If the fluid flows out too rapidly its escape may be temporarily stopped by partly reintroducing the plunger into the needle. The small size of the needle will avoid too much rapidity in the removal of the ventricular fluid and injection of serum as well as unnecessary trauma of the brain tissue. After withdrawing the fluid from the ventricle a gravity outfit, consisting of the barrel of a 10 to 20 c.c. syringe and connecting rubber tubing, is attached by an assistant to the needle and the antimeningitis serum, previously warmed to body temperature, is slowly injected. Care should be taken to inject less fluid than has been removed. At the end of the operation the needle is gently withdrawn and a sterile dressing applied. It is not necessary or advisable to wash out the ventricle with salt solution before the injection of the serum. In repeating the injection it is preferable to use the other ventricle. Each specimen of fluid withdrawn is carefully examined by smears and bacteriological cultures.

The infant should be kept in the hospital under observation for at least four weeks after convalescence has set in. Before discharging the patient a lumbar puncture should be made to see whether a clear spinal fluid can be obtained. This would indicate the reëstablishment of the communication through the foramina of Magendie and Luschka between the ventricular cavities in the brain and the subarachnoid space in the spinal canal.

Every effort should be made to have breast feeding continued during the patient's stay in the hospital. This is an important feature of the treatment, as changing to bottle feeding will greatly diminish the chances of the infant's recovery. After the patient has been discharged from the hospital, observations should be continued for a period of several years to determine any possible impairment of the mentality or of any of the special sense organs.

The following are the records of the two cases treated at the Willard Parker Hospital. The contrast in the final results, due to the institution of proper treatment at different stages of the disease, illustrates quite definitely the necessity of early ventricular puncture for diagnosis and treatment.

Case I.—Frank S. (Chart I.) Patient, aged two and a half months, was admitted to the hospital on May 9, 1917. Had been ill for eighteen days previous to admission. The patient presented the typical clinical picture of an advanced basilar meningitis. There was marked retraction of the head, opisthotonos, spasm of the limbs and bulging of the anterior fontanelle. The patient was first seen at home by a physician connected with the meningitis division of the Department of Health. A lumbar puncture was made but it resulted in a dry tap. Two other punctures were made at subsequent visits, but each time only a few drops of sterile bloody fluid were obtained. After admission to the hospital on May 9 (nineteenth day of disease) four more lumbar punctures were made, which again only resulted in a few drops of sterile spinal fluid. At each lumbar puncture a few cubic centimeters of antimeningitis serum were injected intraspinally.

The patient showed no clinical improvement during this time. His condition became rapidly worse; the opisthotonos was more marked, the anterior fontanelle showed greater bulging and the child was becoming progressively more emaciated.

May 15 (twenty-fifth day of disease). First ventricular puncture was made; 45 c.c. turbid fluid obtained, containing numerous meningococci in smears and cultures; 20 c.c. antimeningitis serum injected by gravity.

May 16 (twenty-sixth day of disease). Anterior fontanelle bulging. Second ventricular puncture; 45 c.c. turbid fluid withdrawn, showing several colonies in culture; 20 c.c. antimeningitis serum injected.

May 17 (twenty-seventh day of disease). Third ventricular puncture; 40 c.c. slightly cloudy fluid withdrawn, which showed only one colony of meningococci; 18 c.c. antimeningitis serum injected.

May 18 (twenty-eighth day of disease). Fourth ventricular puncture; 10 c.c. slightly cloudy fluid obtained, which was sterile in culture. No serum injected.

May 20 (thirtieth day of disease). Fifth ventricular puncture; 30 c.c. almost clear fluid obtained, which was sterile in culture; 20 c.c. antimeningitis serum injected by gravity.

May 21 (thirty-first day of disease). Patient died. Toward the end the patient showed marked marasmus.

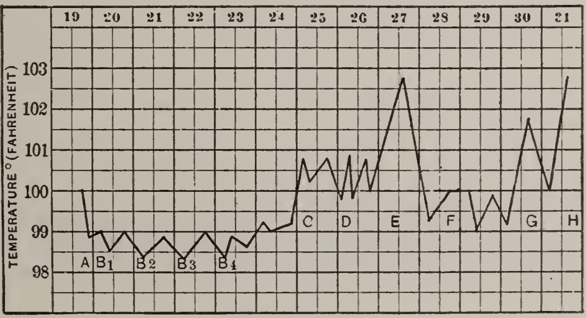

CHART I.—Frank S. (Case I). *A,* lumbar puncture; 15 c.c. blood-tinged fluid withdrawn; *B*1, lumbar puncture; few drops of blood-tinged fluid withdrawn; *B*2, lumbar puncture; few drops of blood-tinged fluid withdrawn; *B*3, lumbar puncture; few drops of blood-tinged fluid withdrawn; *C,* first ventricular puncture; 45 c.c. of turbid fluid withdrawn; 20 c.c. serum given; *D,* second ventricular puncture; 45 c.c. of turbid fluid withdrawn; 20 c.c. serum given; *E,* third ventricular puncture; 40 c.c. of slightly turbid fluid withdrawn; 18 c.c. serum given; *F,* fourth ventricular puncture; 10 c.c. of slightly turbid fluid withdrawn; *G,* fifth ventricular puncture; 30 c.c. almost clear fluid withdrawn; 20 c.c. serum given; *H,* patient died.

Case II.—Giulietta M. (Chart II). Two and a half months old. Admitted to the hospital May 13, 1917. Ill for three days before admission. Symptoms slight and not characteristic. Temperature, 100° F.; occasional tremors of upper and lower extremities; slight but distinct bulging of anterior fontanelle even when the baby was quiet. No rigidity of neck or Kernig's sign. On day of admission a lumbar puncture gave 2 c.c. of purulent fluid containing numerous meningococci in smears and cultures. Several other lumbar punctures were made on the same day and on the following morning but no more spinal fluid could be withdrawn nor could the antimeningitis serum be injected into the spinal canal; 20 c.c. of serum were therefore given subcutaneously on the day of admission.

May 14th (fourth day of disease). First ventricular puncture; 15 c.c. of cloudy fluid withdrawn, which showed numerous meningococci in smears and culture; 10 c.c. serum injected.

May 16 (sixth day of disease). Anterior fontanelle bulging; second ventricular puncture made; 20 c.c. turbid fluid obtained, showing a smaller number of meningococci in smears and culture; 10 c.c. serum injected.

May 18 (eighth day of disease). Third ventricular puncture; 40 c.c. slightly turbid fluid obtained, sterile in culture; 15 c.c. serum injected.

May 20 (tenth day of disease). Fourth ventricular puncture; 40 c.c. slightly turbid fluid withdrawn, which was sterile in culture; 20 c.c. serum injected.

May 31 (twenty-first day of disease). Lumbar puncture; 10 c.c. clear spinal fluid obtained, which was sterile in culture. Patient discharged from the hospital.

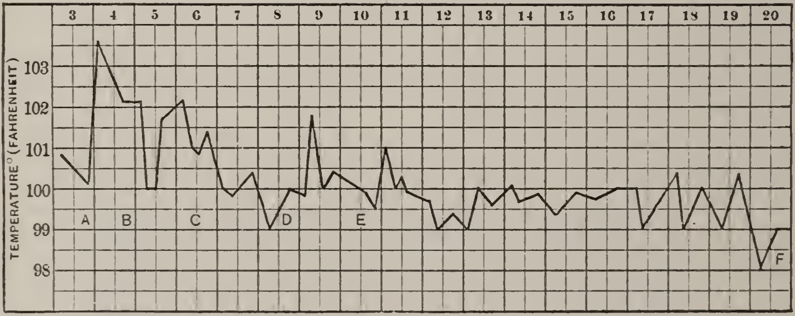

Chart II.—Giulietta M. (Case II). *A*, lumbar puncture; 2 c.c. purulent fluid, containing meningococci; 20 c.c. antimeningitis serum given subcutaneously; *B*, first ventricular puncture; 15 c.c. of turbid fluid withdrawn; 15 c.c. serum injected; *C*, second ventricular puncture; 20 c.c. of turbid fluid withdrawn; 10 c.c. serum injected; *D*, third ventricular puncture; 40 c.c. of turbid fluid withdrawn; 15 c.c. serum injected; *E*, fourth ventricular puncture; 40 c.c. of slightly turbid fluid withdrawn; 20 c.c. serum injected; *F*, lumbar puncture; 10 c.c. of clear spinal fluid, sterile in culture.

During the course of the disease the patient developed a slight retraction of the head, which was hardly noticeable, however, at the time of discharge from the institution. Breast feeding was continued during the entire time. It is interesting to note that a nasal culture made on May 16 (sixth day of disease) showed the presence of meningococci.

An examination made three months after the patient was discharged from the hospital showed that the baby had made a complete recovery.

A reëxamination was recently made (one year after the attack) and the baby was found to be apparently normal and to have made the usual progress in its mental and physical development.

Summary and Conclusion. 1. A prompt ventricular puncture is indicated in cases that show progressive meningeal symptoms and give a dry tap on lumbar puncture. In some patients the lumbar puncture may show a few drops of purulent spinal fluid, but a sufficient amount cannot be withdrawn, even by aspiration, with a syringe, and little or no antimeningitis serum can be injected.

2. Even less pronounced meningeal symptoms, such as slight but definite bulging of the anterior fontanelle, tremors of the extremities and fever should lead to a ventricular puncture in cases that have given repeated dry taps in the hands of an experienced operator. Such early ventricular punctures are of vital importance in the successful treatment of cases of posterior basilar meningitis.

3. In adults the persistence of aggravation of the clinical symptoms, associated with a persistently cloudy spinal fluid, which has become sterile after two or three injections of antimeningitis serum, indicate in many cases the necessity for a ventricular puncture. In these patients the lumbar puncture may show a sufficient amount of spinal fluid, but the outlet from the ventricles is closed off and the serum injected into the spinal canal does not reach the infected regions.

4. Ventricular punctures should be repeated daily or every other day; 20 to 50 c.c. of fluid are withdrawn and 15 to 30 c.c. of serum injected by gravity. The serum should be warmed to body temperature and the amount injected should always be smaller than the amount of fluid withdrawn. Three assistants are generally necessary to carry out the operation carefully and safely.

5. The interval of time between punctures and the total number of punctures will depend upon the rapidity in the reaccumulation of the fluid (indicated clinically by the reappearance of a bulging anterior fontanelle), and upon the result of the bacteriological culture of the ventricular fluid.

6. A lumbar puncture should be made at the time the patient is discharged from the hospital in order to determine the reëestablishment of communication between the ventricular cavities in the brain and the subdural space in the spinal canal.

7. If the baby is breast-fed every effort should be made to have the breast feeding continued while the patient remains in the hospital.

8. The diagnosis may be assisted occasionally in cases giving a dry tap, before deciding upon a ventricular puncture, by making a culture of the nasal discharge and finding the meningococcus present.

9. Cases of posterior basilar meningitis should be followed up for a period of years to determine the final outcome in those who recover after ventricular punctures and injections of antimeningitis serum

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THE DIAGNOSIS AND SERUM TREATMENT OF ANTERIOR POLIOMYELITIS.*

Abraham Zingher

The application of immune serum from human beings who have recovered from the disease has been recommended by Netter[1] for the treatment of the acute stages of poliomyelitis. Netter gives the results of such treatment in a small series of cases. His therapeutic use of the serum was suggested by the earlier work of Römer and Joseph,[2] Landsteiner and Levaditi,[3] and Flexner and Lewis,[4], who detected neutralizing immune substances in the serum of monkeys that had recovered from an attack of poliomyelitis; by the later work of Levaditi and Netter[5] and Flexner and Lewis,[6] who independently showed similar immune substances in the serum of convalescent human cases; and also by the subsequent work of Flexner and Lewis,[7] who showed that immune human serum had the power of preventing the development of the disease in monkeys, even when injected twenty-four hours after the intracerebral inoculation of a fatal dose of virus.

On the basis of these results, the work with immune serum was taken up at the Willard Parker Hospital, at the Minturn Hospital and in the private practice of a number of physicians to whom the serum was supplied for treatment of suitable cases. It was soon realized that cases in the preparalytic stage of the disease would be the most suitable for serum treatment, and that late cases with well developed paralysis and normal temperature would probably not be influenced by the administration of serum. The serum was also used, however, in cases where paralysis had already developed in some parts, and was progresssively involving other members of the body while the patients were still showing a high temperature.

It was in the group of early cases, treated in the premonitory or preparalytic stage of the disease, that we might expect to obtain much more tangible results. In cases showing the development of paralysis after the use of the serum, the following points were noted:

1. The number of doses and amount of serum used each time.
2. The group to which the serum belonged.
3. The number of days the patient had been ill.
4. The number of hours or days which had elapsed since the first dose of serum was given.
5. The degree of paralysis.
6. The rapidity with which the paralytic phenomena began to clear up.
7. The final result: recovery, paresis or paralysis, and the actual groups of muscles affected.

It was only by comparing results in a large group of early cases treated with immune serum, with results given by similar groups who had received normal human serum, or no serum at all, that we could hope to arrive at any certain data as to the efficacy of the serum treatment.

To help in the diagnosis of early cases, the following symptoms and findings in the spinal fluid were found of value.

Symptoms of Early Cases

A certain group of symptoms have been found significant, especially if occurring during an epidemic, in the early stages of infantile paralysis. These symptoms are present in part or in whole in most of the cases. In addition to the history of exposure, which is especially important during an epidemic, we have: (1) fever; (2) hyperesthesia; (3) slight rigidity of the neck, with pain on forward traction, and occasionally a slightly positive Kernig's sign; (4) muscular tremors, irregular from fine to coarse, noticeable especially in the fingers and hands, but also apparent in the entire extremities; (5) weakness of the limbs, especially of the lower extremities, with early diminution or loss of the patellar reflexes; (6) headache; (7) nausea, vomiting and constipation or diarrhea, and (8) the character of the spinal fluid obtained by lumbar puncture in suspicious cases.

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* Published in Jour. Med. Assn., 1917, 68, p. 817.

The macroscopic changes in the spinal fluid to be described cannot replace the more reliable data obtained by careful laboratory examination, but will be found of great value, especially where immediate laboratory facilities are not at hand, and the diagnosis of poliomyelitis has to be confirmed for purposes of isolation and treatment.

Spinal Fluid.

This fluid, with a few exceptions, is quite characteristic even in the early stages of the disease. There is a distinct increase in the total number of cells, from 20 to 600 or more. These cells are mostly lymphocytes, with a small proportion of large mononuclear and polynuclear cells. During the earliest stages of the disease there may be a transient relative and absolute polynucleosis. The chemistry of the fluid is also characteristic. There is an increased amount of albumin, globulin, and a substance which reduces Fehling's solution. Often on the surface of the spinal fluid, as it is being drawn into a test tube, a thin filament of fibrin appears, which breaks up into small flakes when the fluid is gently shaken. The diagnosis in a large percentage of cases can be established in the early cases during the acute stage of the disease, even before the laboratory examination of the spinal fluid, by noting a slight but characteristic opalescence to the fluid which gives a ground-glass appearance throughout the fluid when it is examined in a clean test tube by transmitted light. This appearance is caused by the increased number of white blood cells (lymphocytes), which can be distinctly seen with the naked eye, and still better with a pocket magnifying lens. The large number of lymphocytes appear as dustlike little specks uniformly suspended in the fluid. These particles can be put into motion by a slight shaking movement of the test tube. A poliomyelitis fluid which has been standing for a number of hours, and in which the cells have settled to the bottom, also appears clear unless the cells are distributed again by shaking. A normal spinal fluid looks limpid and gives the appearance of distilled water. The examination of the spinal fluid is best made in a dark room with the test tube held against an artificial light. It is not as accurate a test of cell increase as the exact count, but is of great practical value. Any bedside test by which cases of poliomyelitis can be diagnosed during the early stages is important both for purposes of isolation of the patient and for serum treatment. Several precautions must be observed in utilizing this test. First, there must be no red blood cells in the spinal fluid. Red blood cells, if only few in number, cannot be distinguished macroscopically by the appearance of the fluid, and the opalescence produced by these cells may be mistaken for that caused by white blood cells. When more numerous, however, the red cells are recognized by a characteristic yellowish shimmer to the fluid. A subsequent microscopic examination should always be made to exclude the presence of red blood cells. Second, it must be remembered that white blood cells (lymphocytes and polynuclears) are found increased in the spinal fluid in a variety of other conditions, especially tuberculous meningitis, epidemic cerebrospinal meningitis, syphilitic involvement of the meninges and vessels of the brain, etc. The typical macroscopic ground-glass appearance of the fluid cannot, therefore, be of absolute, but is of great relative diagnostic value during an epidemic, if the precautions mentioned above are observed.

In the later stages of poliomyelitis, generally after the seventh to the tenth day, the cells in the spinal fluid rapidly diminish in number and soon reach a normal count. It is often important to establish the diagnosis in these later stages of the disease, especially when no paralytic phenomena have appeared after the attack. A simple and yet fairly accurate way of diagnosing these cases is to remove the spinal fluid by lumbar puncture and examine its albumin and globulin content. For a period of from eight to ten weeks, a majority of persons who have passed through an attack of poliomyelitis will show an increased amount of both these substances. During an epidemic this is strongly suggestive of an attack of the disease, especially in connection with a history of some or all of the preparalytic symptoms of poliomyelitis.

TABLE 1.

ALBUMIN CONTENT OF SPINAL FLUID IN POLIOMYELITIS

Days Ill.	Total No. Spinal Fluids.	Maximum Amount.		Large Amount.		Moderate Amount.		Slight but Distinct Increase.	
		Fluids.		Fluids.		Fluids.		Fluids.	
		No.	%	No.	%	No.	%	No.	%
1– 7	52	8	15.4	22	42.3	18	34.6	4	7.7
7–14	39	20	51.3	10	25.6	6	15.4	3	7.7
14–21	35	12	34.3	7	20.0	11	31.4	5	14.3
21–28	31	8	25.8	12	38.7	7	22.6	4	12.9
28–35	26	3	11.5	4	15.4	14	53.8	5	19.2
35–42	37	1	2.7	8	21.6	18	48.6	10	27.4
42–49	51	4	7.8	14	27.5	20	39.2	13	25.5
49–56	50	2	4.0	11	22.0	26	52.0	11	22.0

Table 1 shows rather strikingly the persistence of the albumin increase (the globulin is generally in proportion to the albumin content) even as late as eight weeks after the onset of the symptoms. It is interesting to note that the maximum amount of albumin is found in a much larger proportion of cases during the second week rather than during the first week of the disease. It is possible, therefore, to clear up the diagnosis of some of the abortive types of poliomyelitis even during the later stages of convalescence.

In addition to the ground-glass appearance of the fluid, which is a useful test to determine an increased number of cells and of value in the early stages of poliomyelitis, there is a second macroscopic test, of value both early and late in the disease. This is the so-called foam test, which depends on the pathologically increased amount of albumin and globulin in the spinal fluids in cases of poliomyelitis. When a test tube which is half filled with spinal fluid is thoroughly shaken, a persistent foam appears on the surface, which may last for half an hour to an hour or even longer. Compared with a normal spinal fluid, the foam produced by shaking a fluid obtained from poliomyelitis patients is much denser, finer, more voluminous and more persistent. Here, also, the presence of blood must be excluded before any definite deductions from this test are made.

The importance of getting a spinal fluid free from blood is evident from the diagnostic value attached to the fiindings in a clear spinal fluid obtained from early and late cases of poliomyelitis. After using a variety of lumbar puncture needles, I have become thoroughly convinced that the needle for diagnostic puncture should not be larger than a No. 18 guage and not longer than 3 inches. Such a needle is easily handled, does not bend, and causes little trauma and very little pain. With the patient placed in the proper recumbent position, and the back properly arched, the needle is introduced almost vertically to the spine but given a slight upward direction toward the head of the patient, in the median line between the third and fourth lumbar vertebrae on a level with the crest of the ilium. Anesthesia, either local or general, is, as a rule, unnecessary. When the child struggles considerably, and the examination of the spinal fluid is of great importance in clearing up the diagnosis, a light ether anesthesia for a few minutes is justifiable and indicated.

Serum Treatment

The diagnosis in the early cases having been established by clinical symptoms and examination of the spinal fluid, the next step is to inject the serum into the spinal canal. It is here that the bedside confirmation of the diagnosis by the macroscopic appearance of the spinal fluid becomes most important. In cases in which the fluid indicates it, the serum can be promptly administered without any further delay for laboratory examination and without the necessity of another lumbar puncture, although the confirmatory laboratory examination can be made later.

Fifteen cubic centimeters of the serum are injected by the gravity method after the removal of a slightly larger amount of spinal fluid. The dose is repeated every twenty to twenty-four hours until two or possibly three injections have been made. In the more severe cases, especially with an advancing involvement of the respiratory muscles, the serum should be given every twelve hours. In considering the frequency of the repetition of the dose, and especially the interval of time between the injections, it is important to remember what is taking place in the cerebrospinal meninges after an injection of the serum. A marked polynuclear cellular reaction is produced, which probably should be given time to exert its full effect before the spinal canal is tapped again and the rich cellular fluid removed to give opportunity for the administration of a second or third dose of serum. If one delays the reinjection of serum for forty-eight hours longer, there will be noticed a rapid clearing up of the artificially produced cellular increase in the spinal fluid. Considering this therapy as a possible nonspecific cellular stimulation obtained by the injection of a rich protein fluid, we should consider that an interval of from twenty to twenty-four hours between the injections is best; but it is fair to state that in three severe cases the readministration of the serum was made every twelve hours with apparently good results. The serum is rapidly absorbed from the spinal canal, so that the daily repetition of the dose is indicated, especially if the efficacy of the serum is considered as being based in part on its antibody content.

SOURCE OF SERUM AND GROUPS OF IMMUNE SERUM

The immune serum used in the treatment of cases of infantile paralysis was obtained from convalescents and from immune donors who had had the disease from one to several years previously. For the sake of accuracy, and to facilitate the proper study of its action, the serum was classified into groups, acording to the number of years which had elapsed since the immune donors had had the disease. It was important in choosing donors for immune serum to establish the fact that they had really suffered from infantile paralysis, and not to accept the diagnosis of the donor on his word, as cases of Bell's palsy, tuberculous disease of the bones and joints, hemiplegias, syphilitic and otherwise, could be found among such so-called immune donors.

The groups into which the serum was divided were:
Early convalescent serum, from two to six months after an attack.
Late convalescent serum, from six to twelve months after an attack.
Group A Serum, from one to five years after an attack.
Group B Serum, from five to fifteen years after an attack.
Group C Serum, from fifteen to thirty years after an attack.
Group D Serum, from thirty years up after an attack.

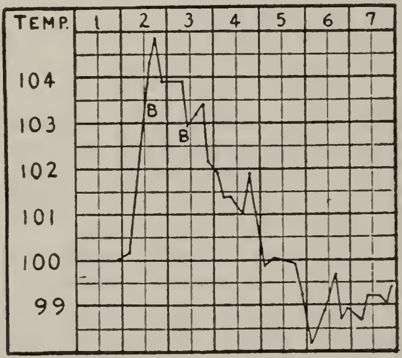

Chart 1.—Typical curve in preparalytic case of poliomyelitis in which immune serum was given and which ended in complete recovery: *B*, injection of 15 c.c. immune serum Group B.

METHOD OF OBTAINING AND PREPARING SERUM.

(*a*) *Amount.*—To obtain the immune serum, the blood is drawn from suitable donors in quantities varying with the age, weight and apparent hemoglobin content of the individual. On an average, it is safe to withdraw 2 ounces from children 9 to 10 years of age, 3 to 4 ounces from children 12 to 13 years of age, and from 4 to 6 ounces from persons 18 years of age and over. Adults, especially robust, full-blooded persons, can furnish from 10 to 16 ounces of blood. Similar amounts of blood can be safely withdrawn again at the end of two to three weeks.

(*b*) *Technic—Obtaining the Blood.*—The blood is obtained by means of a No. 15 gage steel or platinum needle, to which a small piece of rubber tubing is attached. In children, adults and stout persons with small or indistinct veins, the blood is withdrawn by means of a 1 ounce record syringe, and a No. 17 gage needle. The blood is collected in small square bottles in quantities of from 1 to 2 ounces and given a long slant so as to obtain as broad a surface for the separation of the serum as is possible.

(*c*) *Preparaton of the Serum.*—The blood is allowed to clot, and the bottles are placed in the ice box during the following twenty-four hours to allow a separation of the serum. This is decanted the following day and centrifuged to free it from pieces of blood clot and red bloods cells. To the serum is next added a preservative in the form of 0.2 per cent. tricresol. This is added in a 25 per cent. solution in quantities of 4 c.c. to every 500 c.c. of serum. The serum is then allowed to remain in the ice box for forty-eight hours, so that a fine precipitate, which forms after the addition of the tricresol, separates out and is removed by decantation. The serum is then passed through a Berkefeld stone filter, either by suction or by pressure, bottled in quantities of 15 c.c. in dark amber or blue bottles, and kept cold in the ice box. The tricresol was found to increase the local irritant effect of the serum, and it may be advisable not to add it as a preservative.

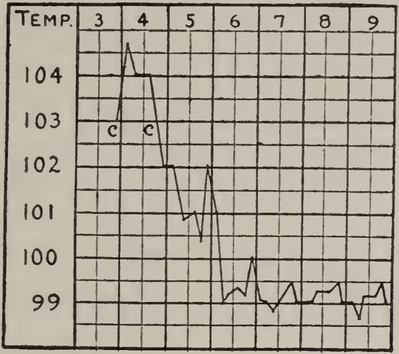

Chart 2.—Typical curve in preparalytic case of poliomyelitis in which immune serum was given and which ended in complete recovery: *C*, injection of 15 c.c. immune serum Group C.

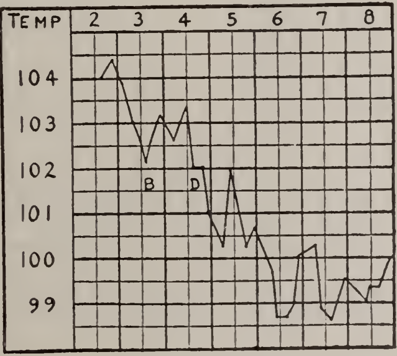

Chart 3.—Typical curve in preparalytic case of poliomyelitis in which immune serum was given and which ended in complete recovery: *B*, injection of 15 c.c. immune serum Group B, D, injection of 15 c.c. immune serum Group D.

(*d*) *Duration of Efficiency of the Serum.*—If the serum has been preserved with tricresol, or handled with sterile precautions after it has been passed through the Berkefeld filter, and is afterward kept in a cold place, it will probably remain efficient in its specific antibody content for a number of weeks. So far the serum has been used up almost as fast as it has been obtained. Some of the serum which had been kept for from four to six weeks seemed to be as active therapeutically as the more recently drawn serum.

In an emergency, or when the facilities for treating the serum are not obtainable, the blood is simply drawn in a perfectly aseptic condition into a vessel with glass beads, shaken and centrifuged; or the serum drawn in the usual way is allowed to separate during the next few hours, and promptly used, the presence of a few suspended red blood cells being disregarded entirely. When immune serum is not obtainable, fresh normal serum is generally procurable from the parents or other relatives of the patient, and can be used within a few hours. Certainly it is advisable not to fail to administer human serum in the treatment of any case of poliomyelitis, because of a lack of already prepared serum.

It is important to note in the preparation of serum, either for stock or to be used fresh without a preservative, that no heat is applied either for inactivation or for sterilizaton.

Each donor should be ascertained to be healthy and to give a negative Wassermann reaction.

Results of Serum Treatment.

The most evident action of the serum was a marked cellular response of the cerebrospinal meninges in the presence of the serum injected by lumbar puncture. This cellular reaction consisted of a very decided increase of the polymorphonuclear cells, which preponderated in some of the cases to the extent of 95 per cent., while the total cell count increased to from 500 to 10,000 cells per cubic centimeter. This increase of cells was at times so pronounced that the spinal fluid obtained at the end of twenty-four hours had a very marked turbid, almost purulent appearance, and a heavy sediment of cells was found in the test tube within a few hours after the lumbar puncture. Culturally, these fluids were always found sterile. This cellular response also followed the injection of normal human serum, of normal horse serum, and of the secondary albumoses of Jobling (used in a few cases) when these injections were given in the same early stages of disease. This high cellular response of the cerebrospinal meninges is probably due to the very marked congestion of the vessels of

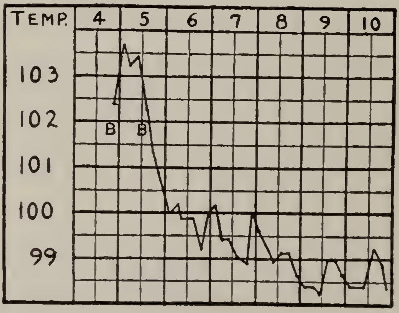

Chart 4.—Typical curve in preparalytic case of poliomyelitis in which immune serum was given and which ended in complete recovery: *B,* injection of 15 c.c. immune serum Group B.

the pia-arachnoid and of the cerebral cortex. It is probable that in the use of immune serum we have, in addition, the helping factors of antibodies against the virus of poliomyelitis which stimulate the polymorphonuclear cells to increased phagocytic activity. We cannot estimate the phagocytic action of the cells, as the virus is too minute to be seen. It must not be overlooked, however, that we have probably obtained therapeutic results as good after the use of serum from Group C or Group D as from Group A or B. Such serums may have only a problematic value as antiserums, and almost their entire action may depend on the rich protein content of all blood serums. Further experimental work will no doubt have to be carried out in monkeys to determine whether the injection of immune serums into the spinal canal of monkeys will have a greater protective value against a previous infection with active virus than the injection of normal human serum.

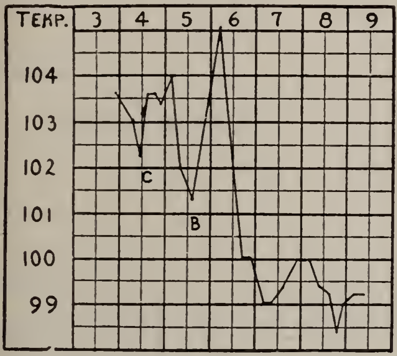

Chart 5.—Temperature curve of a patient with poliomyelitis who developed a facial paralysis eighteen hours after the first dose of immune serum. This patient made a complete recovery: *C*, injection of 20 c.c. immune serum Group C; *B*, injection of 15 c.c. immune serum Group B.

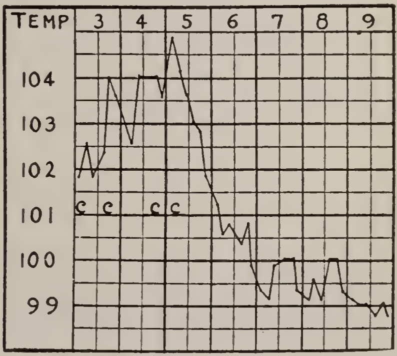

Chart 6.—Temperature curve of a patient with poliomyelitis who developed paralysis of both lower extremities and partial paralysis of both deltoids on the day of the third injection of immune serum. This patient made a partial recovery: *C*, injection of 15 c.c. immune serum Group C.

When the injection of immune or of normal human serum was made in the later stages of the disease, after the temperature had already subsided, the cellular response was found to be much poorer, and only an opalescence of the spinal fluid was noticeable at the end of twenty-four hours. This lessened cellular response is probably due to a subsidence of the acute congestion of the brain and meninges.

Clinically, the injection of either immune or normal human serum was followed within twenty-four hours in the early preparalytic cases by an intensification of the meningeal symptoms. There were increased rigidity of the neck, opisthotonos, marked Kernig's sign, hyperirritability, headache, vomiting and increased temperature which in the most severe reactions reached 104 degrees or 105 degrees F. Occasionally, when the reaction was especially severe, there were twitchings or even convulsive movements of the extremities. These symptoms represented merely a more severe degree of the early preparalytic phenomena of the disease. In some of these cases the temperature was simply caught in its upward rise in the course of the disease, and had, therefore, no significance with regard to the treatment.

Definite temperature reactions were, however, noted after primary and also after secondary injections of serum. The typical curve can be seen in Charts 1 to 4 which show a rise in temperature, persisting for another twenty-four hours, generally without any further increase after a second dose of serum and subsiding by a rapid lysis. Charts 5 and 6 show temperature curves of patients who developed paralysis during the course of treatment.

In the patients recovering after serum treatment, symptoms like rigidity of the neck and Kernig's sign, persisted for from two to three days after the temperature had dropped to normal. Gradually all symptoms cleared up and the patient, if there was no complicating paralysis, made a rapid and uneventful convalescence.

The cases treated with immune serum may be divided into three groups:

1. Cases treated at the Willard Parker Hospital.
2. Cases treated at the Minturn Hospital.
3. Cases treated with other physicians.

1. Willard Parker Cases.—A. *Immune Serum Cases.*— (*a*) Preparalytic Cases: Twenty-five:

Type of Serum		No. of Doses	
A	0	One	20
B	1	Two	4
C	17	Three	1
D	7		
	25		25

Results.—(1) Twenty-four remained free from paralysis; (2) one had weakness of both quadriceps extensor (Serum C); (3) none died.

(*b*) Paralytic Cases: Eighty-eight: 1. Thirty-eight patients died: (*a*) Eighteen died in less than twenty-four hours after first dose of serum; (*b*) nine died within forty-eight hours after first dose of serum; (*c*) five died in more than forty-eight hours after first dose of serum; (*d*) two died of tuberculous meningitis; (*e*) one died of gastro-enteritis, and (*f*) three died of pneumonia.

Type of Serum		No. of Doses		Type of Case	
Convalescent	1	One	23	Bulbar	7
A	4	Two	13	Bulbospinal	6
B	10	Three	2	Spinal	22
C	23			Cerebral	1
				Tuberculous	2
	38		38		38

A large proportion of the fatal cases were in children in the last stages of an advancing Landry type of poliomyelitis. Of the eighteen who died within less than twenty-four hours, a majority were moribund, and passed out within a few hours after the administration of a single dose of serum. Many of the patients in this group received the serum only as a last resort. The bulbar cases had well pronounced respiratory difficulty before the serum was injected.

2. Fifty lived:

Type of Serum		No. of Doses		Type of Case	
Convalescent	1	One	39	Bulbar	8
A	6	Two	9	Bulbospinal	2
B	21	Three	1	Spinal	40
C	20	Four	1	Cerebral	0
D	2				
	50		50		50

These patients at the time of treatment already had a well developed paralysis, but were in a still active stage of the disease, as shown by temperature and a spreading type of lesion. These children recovered and showed a definite clinical improvement; but it is difficult to state how much of the improvement, which is also generally seen in the untreated cases, was due to the administration of the serum, and how much would have taken place during the natural course of the disease.

B. *Normal Serum Cases.*—To determine whether a similar cellular reaction would be produced by the intraspinal injection of normal serum and similar therapeutic results obtained, a series of preparalytic and paralytic cases was treated with serum obtained from persons who had never, to their knowledge, had poliomyelitis. It is, however, recognized that persons who have never had the lesion may have protective substances in their blood.

(*a*) Preparalytic Cases: Ten:

No. of Doses		Results	
One	7	Remained free from paralysis	9
Two	3	Developed bulbar symptoms within 12 hours and died	1
	10		10

(*b*) Paralytic Cases: Thirty-three: 1. Five patients died: (*a*) three died within less than twenty-four hours after first dose of serum; (*b*) one died of sepsis, and (*c*) one died of pneumonia.

No. of Doses		Type of Case	
One	3	Bulbar	1
Two	2	Spinal	4

Two of the children who died within less than twenty-four hours had advanced ascending spinal cases of the Landry type, and one a bulbar case.

2. Twenty-eight patients lived:

No. of Doses		Type of Case	
One	21	Bulbar	5
Two	7	Spinal	23

There was a distinct clinical improvement in the patients who recovered, but whether more than would naturally take place without the use of serum it is difficult to say.

The final results of the Willard Parker Hospital cases were obtained from the hospital records, since I followed up some of the cases for only a short time after the injections of serum.

2. MINTURN HOSPITAL CASES.—(*a*) Preparalytic Cases: Fifteen:

Type of Serum		No. of Doses	
A	0	One	0
B	5	Two	11
C	9	Three	2
D	1	Four	2
	15		15

Results: (1) Nine remained free from paralysis; (2) two developed paralysis within less than twenty-four hours: (*a*) bulbar, with complete recovery (B), and (*b*) facial, with complete recovery (C); (3) four developed paralysis after forty-eight hours: (*a*) facial, with complete recovery (B); (*b*) internal strabismus, with complete recovery (C); (*c*) both lowers and left deltoid, with marked improvement (C), and (*d*) both lowers, both deltoids, with final improvement (C).

This series represents an interesting and valuable group of cases. Each patient was fully treated and carefully observed for a period of eight weeks, and the clinical results lead one to the conclusion that the serum was distinctly beneficial in the treatment of these preparalytic cases.

(*b*) Paralytic Cases: Eighteen:

Type of Serum		No. of Doses		Type of Case	
A	3	One	7	Bulbar	2
B	3	Two	7	Spinal	16
C	12	Three	2		
D	0	Four	2		18

Results: Fifteen recovered with improvement of paralysis; three died: (1) two Landry spinal, and (2) one bulbar.

The cases in this group represent chiefly the usual spinal type, in an active stage of the disease, as shown by the short history and the presence of high temperature. The fatal cases were seen and treated at a time when the prognosis seemed to be very doubtful.

In addition to the serum-treated patients there were at the Minturn Hospital forty one patients who received no serum. The majority of these cases came under observation during the stage of convalescence.

3. Cases Treated with Other Physicians.—These patients were personally observed and treated.

(*a*) Preparalytic Cases: Fourteen. No patients died.

Type of Serum	
A	3
B	2
C	7
D	2
	14

No. of Doses	
One	7
Two	3
Three	4
	14

Results: Eleven remained free from paralysis; three developed paralysis: (1) facial and strabismus with complete recovery (A); (2) anterior tibial groups with marked improvement (D), and (3) paresis of both upper eyelids with recovery (C).

This group of cases treated in the preparalytic stage of the disease is also interesting in the large number of complete recoveries which followed the treatment with immune serum.

(*b*) Paralytic Cases: Thirteen:

Type of Serum	
A	1
B	3
C	8
D	1
	13

No. of Doses	
One	6
Two	2
Three	5
	13

Type of Case	
Bulbar	2
Bulbospinal	1
Spinal	10
	13

Results: Nine patients recovered with improvement in paralysis; four died: (1) three Landry spinal, and (2) one bulbar.

The bulbar patient died within two hours. It was a far advanced case, and the prognosis was poor. Of the three Landry spinal patients, two died within less than twenty-four hours after treatment.

In addition to these cases that were seen personally, the serum was supplied to physicians for over 200 cases. The reports obtained from the physicians are not complete, but the cases reported indicate results similar to those detailed above.

4. Control Cases.—It is very difficult indeed to state definitely to what extent the results obtained with serum could be ascribed to the action of the serum alone, either as a specific or as a nonspecific form of treatment. As stated above, the natural course of the disease is variable. For purposes of control, therefore, a series of twelve nontreated preparalytic cases was taken without selection from the records of the Willard Parker Hospital.

Control Preparalytic Cases: Twelve: (*a*) Five remained free from paralysis; (*b*) seven developed paralysis: (1) bilateral ptosis with little movement of eyeballs; (2) complete facial paralysis; (3) paresis of right arm and left leg; (4) paresis of right leg; (5) paresis of neck muscles; (6) paresis of both quadriceps extensor; (7) paralysis of both lower extremities.

At the Minturn Hospital there were three nonparalytic patients, admitted eight days after the onset of the symptoms, who remained free from paralysis. Of two preparalytic patients, one developed strabismus and one paresis of both lower extremities.

Table 2 gives a summary of the cases treated with immune and with normal human serum. It is interesting to see that of fifty-four preparalytic cases treated with immune serum, forty-four remained free from paralysis, while of the ten who developed some form of paralysis, five made a complete final recovery. The results with normal serum seem to be very favorable, but the number of cases treated in the preparalytic stage of the disease is too small, and a larger series of cases should be treated before final deductions are made. The high mortality among the paralytic cases is explained by the desperate condition of many of the patients at the time the treatment was administered.

TABLE 2.—SUMMARY OF CASES.

Institution.	Total.	1. Preparalytic.				2. Paralytic.			
		No Paralysis.	Paralysis with Final Recovery.		Died.	Total.	Recovered.		Died.
			Complete.	Partial.			Complete.	Partial.	
Treated with (a) immune human serum:									
Willard Parker Hospital	25	24	..	1	0	88	5	45	38
Minturn Hospital	15	9	3	3	0	18	2	13	3
With other physicians	14	11	2	1	0	13	1	8	4
Total	54	44	5	5	0	119	8	66	45
Treated with (b) normal human serum:									
Willard Parker Hospital	10	9	..	..	1	33	4	28	5

COMMENT

It is known that in poliomyelitis we have a group of abortive non-paralytic cases in patients who go through the premonitory symptoms, but do not develop paralysis. It is difficult to state, therefore, how many of the patients treated with serum would have remained free from paralysis without serum treatment. Conclusions based on any form of treatment in a disease which is so variable in symptomatology and in prognosis, both as to life and disability, must be given with reserve. It seems to me, however, that the action of serum in poliomyelitis is beneficial, that human serum is indicated in the treatment of the acute stages, especially in the pre-paralytic period of the disease and that the use of serum from recent immune donors is preferable to normal serum. When carefully observed and controlled, the treatment is harmless. A distinct reaction follows in some of the cases after the first dose; the reaction is less marked after the second, and little, if any reaction is noted after the third injection of serum.

In two groups of poliomyelitis cases an unfavorable prognosis seemed to be indicated even in the earlier stages of the disease. One group was of the fulminating bulbar type with a rapidly progressive involvement of the respiratory center. The second group, consisting of patients in whom the disease took the form of spinal paralysis was of a type which stood out prominently because of the difficulty in diagnosis, the acuteness of the course of the disease, and the hopelessness of any form of treatment. The type referred to is one which begins with symptoms of rapid respiration which suggest for a few hours the diagnosis of pneumonia. The paralytic phenomena, however, soon became manifest and rapidly spread, involving all the extremities and resulting fatally within from twelve to eighteen hours after the onset of respiratory symptoms. The clinical picture suggests an overwhelming involvement of the cerebro-spinal axis, in which the intercostal and other accessory muscles of respiration, because of their vital functional activity, show the earliest paralytic symptoms.

In the later cases, which were treated after the paralysis had already made a distinct headway and was beginning to involve the muscles of respiration, the serum showed possibly in a certain proportion of cases an inhibitory effect on further progress, which resulted in a saving of life. It is difficult to forecast, however, what the natural result of the disease would have been in these cases. Control patients without serum, in whom the muscles of respiration were involved at the time the patients came under observation, have also recovered. It seemed, however, that a larger number of serum-treated patients of the severe cases had remained alive than would have done so without the use of serum. No very definite judgment of the value of serum can be based on the results obtained in these cases, yet as a possible live-saving procedure, we should continue to use the serum even in unfavorable cases hoping in the course of time to obtain more definite results.

SUMMARY AND CONCLUSIONS

1. The injection of immune and of normal human serum into the spinal canal during the acute febrile stage of poliomyelitis causes a distinct cellular reaction which is mostly polynuclear in type.

2. The increased phagocytic action thus induced is beneficial in poliomyelitis.

3. Such action is enhanced in immune serum by the presence of specific antibodies.

4. It is preferable to use fresh serum, or serum which has been obtained under sterile precautions, passed through a Berkefeld filter and bottled without the addition of a preservative.

5. The presence of a preservative and of hemoglobin in serum increases its irritating effect, and gives rise to the more severe types of reaction.

6. For purposes of treatment, it is important to diagnose the cases during the preparalytic stage of the disease by the typical group of early symptoms and by the changes in the spinal fluid.

7. The characteristic symptoms are fever, slight rigidity of the neck, jerky movements and fine tremors of fingers and hands.

8. The diagnosis of poliomyelitis can generally be confirmed in the early stages of the disease by a bedside examination of the spinal fluid which shows macroscopically the ground-glass appearance and a positive foam test. The laboratory examination will show an increased number of lymphocytes, an increased amount of albumin and globulin, and a marked reduction of Fehling's solution.

9. Parents should be instructed to recognize the early symptoms so as to notify promptly the family physician. This is especially important in detecting secondary cases in the same family.

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INTERPRETATION OF THE SCHICK TEST, THE PSEUDO-REACTION AND THE CONTROL TEST WITH RESULTS OF THE SCHICK TEST IN RECRUITS FOR THE NATIONAL ARMY.*

ABRAHAM ZINGHER.

For the past two and one half years, during which the work with the Schick Test had been carried on by us at Willard Parker Hospital and various other institutions, we have made an attempt to carry out control tests in as many as possible of the cases giving pseudo and combined reactions with the Schick test.

The pseudo-reaction in the Schick test is caused, not by the soluble diphtheria toxin, but by the protein substance of the diphtheria bacillus which is present in the fluid used for the test. The reaction may be obtained in individuals who have enough natural antitoxin to render them immune to diphtheria. It is important, therefore, to control these pseudo-reactions, which are quite frequent in adults, especially in women (from twenty to thirty per cent.). Such pseudo-reactions are, however, relatively rare in children; consequently in making the Schick test on children under four years of age the work can be much simplified by omission of the control test and by making only one observation at the end of seventy-two or ninety-six hours. When the Schick test is to be followed by active immunization with toxin-antitoxin the control test must be made in order to exclude those giving a pseudo reaction but not a positive Schick test. Such individuals should be carefully excluded from the number of those who are to receive immunizing injections, since they are already immune, and also because they are likely to have severe local and constitutional reactions if injected with toxin-antitoxin. In children giving a combined (pseudo-positive) Schick test the immunizing injections must be given and the liability to a disturbing constitutional reaction must be disregarded, as the danger from diphtheria in susceptible children at this age is very great.

For the control tests we at first used a solution containing the autolyzed substance of the Klebs-Loeffler bacillus, since we were convinced of the fact that the pseudo-reaction is a local anaphylactic response to the protein of the diphtheria bacillus. The autolysate was made from a forty-eight-hour agar growth of the diphtheria bacillus (No. 8, Park and Williams), suspended in salt solution in the proportion of ten cubic centimeters of saline to the growth from one Blake bottle of agar. The bacterial suspension was washed four times with physiological salt solution, made up to its original volume with distilled water, then shaken for two hours, killed by heating at fifty-seven degrees centigrade for forty-five minutes and allowed to autolyze in the thermostat over night. The solution was then passed through a Berkefeld filter, and the filtrate so obtained was regarded as a concentrated autolysate. Ten cubic centimeters of this autolysate, representing the growth of one Blake bottle, when injected subcutaneously into a guinea pig, did not show any evidence of soluble toxin. When the unfiltered bacterial suspension was injected into a guinea pig a small knoblike induration developed which soon disappeared.

For use in the control of the Schick test in human beings the concentrated autolysate was diluted one to twenty with salt solution and two-tenths of one cubic centimeter was injected intradermally. Typical false reactions were obtained in the patients giving pseudo and combined Schick reactions but none in the negative or positive Schick cases. By using a more concentrated solution of the autolysate a faint protein reaction could be obtained even in individuals reacting negatively to the smaller amount of protein in the Schick test.

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*A combination of two articles published in: Jour. Am. Med. Assn., 1916, 66, p. 1617; Ibid. 1918, 70, p. 227.

The difficulty of estimating the amount of bacillus protein in the diphtheria toxin broth used for the Schick test, which would serve as a guide in making a proper dilution of the autolysate, prompted us finally to abandon the autolysate as a suitable control substance. We used instead either a toxin slightly over-neutralized with antitoxin, or else a heated toxin in a dilution of the same strength as that of the unheated toxin used in the Schick test. In the over-neutralized toxin (two units of antitoxin to each L + dose of toxin), there was present however, in addition to the toxin, another element, the antitoxin. This, though in extreme dilution (one part to a million as finally injected), might yet be objected to as a possible additional source of error. Therefore we finally selected *heated toxin* as representing probably the most accurate medium for obtaining a proper control test. A dilution of the protein substance of the diphtheria bacillus is thus obtained which is approximately the same as that present in the unheated toxin used for the Schick test, for while the soluble toxic fraction is destroyed by the heating the autolyzed protein is not appreciably affected. To allow, however, for any slight deterioration of the protein we later modified our technique still further by using 25 per cent. more of the heated toxin in our control tests.

The Research Laboratory of the New York Department of Health is supplying an outfit for the Schick test, and another outfit for the control test, together with directions for use.

The outfit for the *Schick* test consists of a capillary tube containing an amount of undiluted ripened diphtheria toxin, which is a little over one minimum lethal dose for a guinea pig, a ten cubic centimeter bottle filled with sterile normal salt solution and a small rubber bulb for the purpose of expelling the contents of the capillary tube into the bottle of saline. In this way a dilution is obtained of which every two-tenths of a cubic centimeter represents one-fiftieth minimum lethal dose of toxin, the amount required for the test. Thirty-five or more Schick tests can be made with the above ten cubic centimeters of dilution, the balance being consumed in unavoidable waste.

The outfit for the *control* test consists of a capillary tube containing diphtheria toxin which has been heated for ten minutes to 75 degrees C. and in which the toxic element has thus been destroyed while the protein has been very little affected. The other articles are the same in the two outfits.

Recent work has shown that the final dilution of heated toxin will remain as potent as the undiluted with regard to its protein content. This holds true for a period of two years, making it conveniently ready for the control test. To popularize the control test, which is so important in enabling us to identify accurately many doubtful reactions, the Research Laboratory will soon furnish a combined package, which will contain the material for the Schick test as stated above, and in addition a bottle of heated toxin of the proper final dilution for the control injections.

The directions for using the outfits are as follows:

Break off one end of the capillary tube, push the broken end carefully through the neck of the rubber bulb until it punctures the diaphragm and enters the cavity of the bulb. Then break off the other end of the tube, hold the bulb between the thumb and middle finger, place the index finger over the opening in the larger end of the bulb and expel the toxin into the salt solution. Rinse out the capillary tube by drawing up the solution several times, then cork the bottle and shake the contents thoroughly.

To make test, inject two-tenths of one cubic centimeter intracutaneously on the flexor surface of the forearm or arm. It is a good plan to use the right arm for the Schick test and the left arm for the control test.

The reactions at the sites of injection should be observed at the end of forty-eight and ninety-six hours. Where only one reading can be made, that should be at the end of seventy-two or ninety-six hours.

The first lesion to appear will be the *pseudo reaction* caused by the protein of the diphtheria bacillus in persons sensitive to this substance. Since the protein is present in both the heated and the unheated toxin the pseudo reaction will be evident at the site of the Schick test as well as that of the control test. It begins at the same time in both locations and runs the same clinical course. In six to eighteen hours there is a beginning redness, which reaches its height at twenty-four to thirty-six hours from the time of inoculation when the lesion appears as a central area of redness of varying size, surrounded by a secondary areola which shades off into the surrounding skin. On the third or fourth day the redness disappears, leaving a poorly defined area of pigmentation, which generally shows very slight or no scaling.

The *positive reaction* begins to appear within 24 hours and reaches its height on the third or fourth day after inoculation, at which time it consists of a definite circumscribed area of redness from one to two and one-half centimeters in diameter. It begins to fade after 7 days and then shows superficial scaling and brownish pigmentation, which persists in some cases for months.

In patients giving a *combined reaction* there is first the pseudo-reaction appearing as described above, at the site of both inoculations. Following this, at the proper interval (about the fourth day, by which time the pseudo element of the test will have disappeared), the positive Schick reaction becomes clearly defined at the site of the Schick test.

In those giving a *negative* test there is no reaction at the site of either the Schick test or the control test.

Individuals showing positive and combined reactions are susceptible to diphtheria, while those giving pseudo and negative tests are immune to this disease.

During the past four years an increasing number of hospitals and institutions have adopted the Schick test as a routine procedure for determining the susceptibility of children and adults to diphtheria.

Occasional outbreaks of diphtheria in military camps and the possibility of preventing them by utilizing the more recent advances in the prophylaxis against this disease caused us to attempt to apply the Schick test to all the recruits for the National Army on admission to Camp Upton, Yaphank, Long Island. Owing, however, to the assignment of the recruits within forty-eight hours to different regiments scattered over the camp, it has been necessary to omit making the tests at the time of the primary physical examination. The plan is to apply the test later, when it can be more conveniently done, to all the members of the regiment. Thus far over two thousand five hundred men have been tested.

The Schick test was made in the usual way on the right forearm, and at the same time the test for the pseudo reaction was made on the left forearm. The reactions were read twice, once at the end of forty-eight hours and again at the end of ninety-six hours. As the pseudo reaction develops earlier and fades more rapidly than the true reaction the early reading is quite important for its identification. The scheme given in Table 1 will show the appearance of the reactions at the two observation periods.

TABLE 1.

APPEARANCE OF THE REACTIONS.*

Time.	Positive. Rt.	Positive. Lt.	Pseudo. Rt.	Pseudo. Lt.	Combined. Rt.	Combined. Lt.	Negative. Rt.	Negative. Lt.
Forty-eight hours	±	—	+	+	+	+	—	—
Ninety-six hours	+	—	$\underline{\pm}$	$\underline{\pm}$	+	$\underline{\pm}$	—	—

*The signs +, ±, $\underline{\pm}$ indicate varying degrees of redness.

TABLE 2.

Table 2 shows the results noted among 2,648 recruits for the National Army.

RESULTS OF THE SCHICK TEST AT CAMP UPTON, N. Y.

Schick Test.	Total.	Per Cent.
First Increment 1,165 Recruits		
Negative	848	72.8
Pseudo	143	12.2
	991	85.0
Positive	154	13.3
Combined	20	1.7
	174	15.0
Second Increment 1,483 Recruits		
Negative	1,072	72.3
Pseudo	143	9.7
	1,215	82.0
Positive	262	17.6
Combined	6	.4
	268	18.0

It is interesting to note in the first increment that the number of pseudo-reactions is about the same as that of the positive reactions. In this group the readings were made at the end of forty-eight and ninety-six hours. In the second increment the number of pseudo-reactions is smaller, that of the positive reactions larger. This is explained by the fact that owing to the rapid assignment of the recruits to the different regiments it was often difficult to take more than one reading and that one in many instances not before the fifth or sixth day. In this way some of the pseudo-reactions were missed altogether.

It will not be difficult to carry out the work systematically among the recruits after the men are grouped in units. The regimental surgeons should be able to make the tests, read the reactions, and have the results noted on the service record of each recruit. Such a record is of value, since it is probable that the individuals who give a negative Schick reaction or a pseudo reaction are protected from diphtheria for several years and possibly for life. Those who show a positive or combined reaction are the only ones that need to be immunized with a dose of antitoxin during an outbreak of diphtheria. These individuals should be actively immunized with diphtheria toxin-antitoxin, so as to develop a lasting protection against the disease.

Conclusions:

1.—In adults the Schick test should always be accompanied by a control injection of heated toxin in order to note the occurrence of an anaplylactic or (pseudo-reaction) due to the protein substance of the diphtheria bacillus present in the fluid used for the test.

2.—The amount of heated toxin in the control fluid should be 25 per cent. more than the amount of unheated toxin in the test fluid.

3.—The making of the Schick test on soldiers gathered in camps is a practical procedure and can be accurately interpreted by using a simultaneous control test with heated toxin.

4.—Observations should be made at the end of forty-eight and ninety-six hours, and the final results noted. In the case of soldiers these data should be put on the service records.

5.—Those giving a positive Schick reaction should be actively immunized with diphtheria toxin-antitoxin.

A MODIFICATION OF ROEMER'S INTRACUTANEOUS METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF DIPHTHERIA ANTITOXIN IN BLOOD SERA.[1]

Abraham Zingher.

It is important for a number of reasons to have a convenient, practical and economic test for the determination of the diphtheria antitoxin content of blood sera. Such a determination would help to give us a solid scientific foundation in many experimental and clinical problems connected with the subject of diphtheria. Among such problems may be mentioned the quantitative estimation of the efficacy of active immunization with toxin-antitoxin, the development of an active immunity after an attack of diphtheria, the antitoxin content of the blood at varying intervals after a single therapeutic or prophylactic injection of diphtheria antitoxin, the effect of one injection of antitoxin on the duration of immunity conferred by a second injection, etc.

The older subcutaneous test of Ehrlich for determining the antitoxin content by the death of the guinea-pig has been universally accepted as a standard, but it cannot be used with any degree of accuracy for the determination of less than 1/20 of a unit of antitoxin to the cubic centimeter of serum. Another objection to the method is that it requires a large amount of serum for each test, especially when the test is made for a fraction of a unit of antitoxin. This large amount of serum is not always obtainable in the case of human beings, especially when we are dealing with small children. The subcutaneous test is expensive also in that it requires the use of one guinea-pig for each test. For these reasons a method which would enable us to determine accurately as little as 1/200 unit of antitoxin would offer many advantages—especially if such procedure required but a small amount of serum and few animals. When many sera are to be tested for small amounts of antitoxin, the intradermal method is practically the only feasible one.

While the negative Schick reaction indicates the development of 1/30 of a unit (according to our work, 1/100 unit) or more of antitoxin to the cubic centimeter of blood serum, it does not show the exact amount of antitoxin that has been produced in the individual. Recent work in active immunization against diphtheria has shown successful results in producing an antitoxic immunity in 90-95 per cent. of susceptible individuals. This was proved by negative Schick reactions in individuals who had previously given positive reactions. Clinically it is possible to demonstrate the continuation of this immunity by repeating the Schick test at definite intervals; but for calculating the probable duration of an immunity it is important according to von Behring to establish quantitatively the amount of antitoxin produced in the actively immunized persons.

In 1909 Römer and Sames[2] published a method for testing small amounts of antitoxin in blood sera. This test was based on a previous observation made by Römer[3] that 1/500 M. L. D. of a toxin was still capable of producing a distinct local necrosis when injected intradermally into the guinea pig.

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[1] Published in Jour. Infect. Dis., 1916, 19, p. 557.

[2] Ztchr. f. Immunitatsf., 1909, 3, p. 49.

[3] Ibid., p. 208.

Römer and Sames determined the necrosing dose of a toxin in the guinea pig, that is, the amount of toxin added to one unit, or any fraction of a unit of antitoxin, which is still capable of producing a slight local necrosis. This was called the Ln dose of the toxin. They found that 1/500 M. L. D. of the toxin was sufficiently neutralized by 1/40,000 unit of antitoxin to just produce a slight local effect in the animal. This amount of antitoxin in 1/20 c.c. of undiluted serum (the quantity used in the test with an equal volume of toxin solution) represents 1/2,000 unit of antitoxin to the cubic centimeter.

The method suggested by Römer and Sames and later applied by Römer and Somogyi [4] to the testing of concentrated antitoxin horse sera, has worked satisfactorily in their own hands, but has not given reliable results in the hands of others who have tried it repeatedly. One of the difficulties lies in the attempts of Römer and Sames to make too fine distinctions between the various lesions of closely lying tests. They claim to be able to distinguish a 5 per cent. difference in antitoxin content between the individual tests. After repeated trials this has seemed to me difficult and unnecessary, especially when we are dealing with an antitoxin content between 1/200 unit and 10 units to the cubic centimeter of serum. A second difficulty and an equally unnecessary point is the long time, twenty-four hours, that the toxin and serum are required to stand before injection into the animal. Thirty minutes has proved to be a sufficient time to allow for the full union between toxin and antitoxin, even in the dilutions used in the test. A third difficulty lies in the necessity of establishing a new test dose, the Ln. These factors have undoubtedly discouraged the use of a test which is inherently valuable.

The modification which I have found simple and workable in the testing of many hundreds of human and animal sera, has the following advantages:—

(1) The L+ of the toxin is taken as the test dose and so diluted that each cubic centimeter represents 1/100 L+ dose.

(2) Two test points are established, one at which no lesion develops and one at which a distinct lesion appears. The amount of antitoxin tested for in the second case must be twice that tested for in the first case. Within these two limits lies the antitoxin content of the serum. The first test indicates a balanced combination or a slight excess of antitoxin, while the second shows the presence of a trace of free toxin. The latter test which produces a slight local necrosis represents the antitoxin content of the serum. When the necrosis is more marked, an intermediate test is made. Example: Slight necrosis with 1 unit, no necrosis with 1/2 unit—Antitoxin content 1 unit. If necrosis were marked, the test would be repeated for 3/4 unit.

(3) The method saves animals in that 4 tests can be made on each guinea pig. It also saves time, for the tests can be interpreted in from forty-eight to seventy-two hours.

(4) As little as 1/200 unit of antitoxin can be determined in a serum with a fair degree of accuracy. If a distinct lesion appears at this test point, the serum is considered as having no antitoxin.

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[4] Ztschr. f. Immunitatsf. 1909, 3, p. 433.

. Details of the Test.

(1) A standard well-ripened toxin is used, so diluted with normal salt solution that 1 c.c. represents 1/100 L + dose. A fresh dilution is prepared for each set of tests. If, for example, the L + is 0.5 c.c. then that amount is diluted with 99.5 c.c. of sterile salt solution. The quantity of toxin used in the test varies with the antitoxin content for which the test is made (see table I).

(2) The serum to be tested is used either undiluted or diluted 1:10 (0.2 c.c. serum + 1.8 c.c. salt solution), 1:100 (0.2 c.c. of serum dilution 1:10 + 1.8 c.c. salt solution), etc. The amount of serum or serum dilution used in the test is always 0.2 c.c. except when the test is made for 1/200 unit of antitoxin; then 0.4 c.c. of undiluted serum is added to 0.2 c.c. of toxin solution.

(3) Normal salt solution is added to balance the mixture. The amount is the same as that of the toxin solution minus 0.2 c.c., which represents the serum, or serum dilution.

(4) After the addition of toxin, serum and salt solution, the mixture is allowed to stand for thirty minutes at room temperature before it is injected into the guinea-pig. This time is utilized in preparing and tagging the guinea-pigs.

(5) The guinea pigs (300-350 gm.) are prepared by removing the hair from the abdomen. This is best accomplished by simply pulling it out, a central line of hair being left to divide the abdominal surface into halves. The pulling of the hair is easily and rapidly carried out, and is probably less painful than the action of a depilatory like calcium hydrosulfid or shaving of the hair. The first occasionally leads to local eczema, the second to abrasions that interfere with the reading of the test.

(6) The animal holder is simply an elevated board with 4 openings for loops of cord with which to fasten the limbs of the animal. This simple device is essential in accurate work and facilitates the exact placing of the injections.

(7) A 1 c.c. "Record" or tuberculin syringe and a fine steel (No. 26 gauge) or platinum-iridium needle are necessary.

(8) Four injections are made into each guinea-pig, the abdomen being for this purpose divided into right upper (R.U.), right lower (R.L.), left upper (L.U.), and left lower (L.L.), quadrants. The injections are made as far apart as possible to avoid a fusion of the lesions. Two-tenths cubic centimeter of each test mixture is injected intradermally. Care with each injection is essential if uniform results are to be obtained. A good guide in the insertion of the needle into the proper layer of the skin is to be able to see its oval opening through the superficial layers of the epidermis. If the fluid has been injected properly, a tense local sharply circumscribed swelling appears, which shows the prominent openings of the hair follicles.

(9) The places of injection on the guinea-pigs are examined at the end of one, two, three and four days, and a careful record is kept of each test (Table 3). The following changes in the local appearance of the skin at the sites of injection are noted:

(*a*) Redness, whether marked (R) or slight (r).
(*b*) Induration, whether marked (Id), moderate (mod) or slight (sl).
(*c*) Necrosis, marked (N) or slight (n), or only superficial scaling (scal).

The induration is most marked at the end of two or three days; the necrosis appears at the end of three days.

In this connection it is important to state that owing to the irritant effect of a foreign protein, some normal human sera will occasionally produce a local lesion when injected into the skin of guinea-pigs even without the addition of toxin. Such a lesion will develop whether the serum is used undiluted or diluted with an equal amount of salt solution; with higher dilutions of the serum, however, no lesion will appear. If, therefore, an antitoxin containing serum is tested for 1/100, 1/50, 1/20 and 1/10 unit, there may develop a lesion at the site of the test for 1/100 unit and no lesions at the site of the tests for 1/50, 1/20 and 1/10 unit. This is because the serum is more highly diluted in the latter tests. These peculiar lesions are quite different from those produced by free diphtheria toxin. They are more circumscribed, nodular, do not spread with the formation of oedema and do not go on to the development of well defined necrosis and scab formation. One doing this work soon comes to recognize lesions due to serum alone and to disregard them. Otherwise in making tests for 1/100 and 1/200 unit, controls would have to be made on the same guinea-pig using serum diluted in the same proportion with normal saline instead of with toxin dilution. A lesion appearing both at the site of the control and of the test injection would further indicate that the action is due to the irritant effect of the serum and not to the free toxin in the test.

The following tables show (1) the amounts of toxin, serum, and salt solution used in the test; (2) the method followed in the examination of several sera; (3) a sample page of the daily record of the tests on the guinea-pig; and (4) an experiment on 4 horses showing the development of antitoxin after injections of small amounts of diptheria toxin-antitoxin.

TABLE I.

INTRADERMAL ESTIMATION OF DIPHTHERIA ANTITOXIN.

Mixtures.			Amount of Antitoxin tested for with Serum.				
Test Toxin 1cc.=1/100 L+	Normal Salt Solution.	Amount of Serum or Serum Dilution.	Undiluted.	Diluted 1:10	Diluted 1:100	Diluted 1:1000	Diluted 1:10,000
0.2 cc.	0.0	0.2 cc.	0.01 unit	0.1 unit	1 unit	10 units	100 units
0.4 cc.	0.2 cc.	0.2 cc.	0.02 unit	0.2 unit	2 units	20 units	200 units
1.0 cc.	0.8 cc.	0.2 cc.	0.05 unit	0.5 unit	5 units	50 units	500 units
2.0 cc.	1.8 cc.	0.2 cc.	0.10 unit	1.0 unit	10 units	100 units	1000units
Every 0.2 cc. of dilution is equal to..........			0.01 unit	0.1 unit	1 unit	10 units	100 units

TABLE II.

Estimation of Diphtheria Antitoxin by the Intradermal Method.

Rectangles indicate blood sera and the dilutions injected.

Circles indicate results of tests. { White—no lesions. Shaded—slight local inflammation. Black—local necrosis.

Figures at side indicate amount of antitoxin tested for.

Other figures indicate result of test in each pig (amount of antitoxin found).

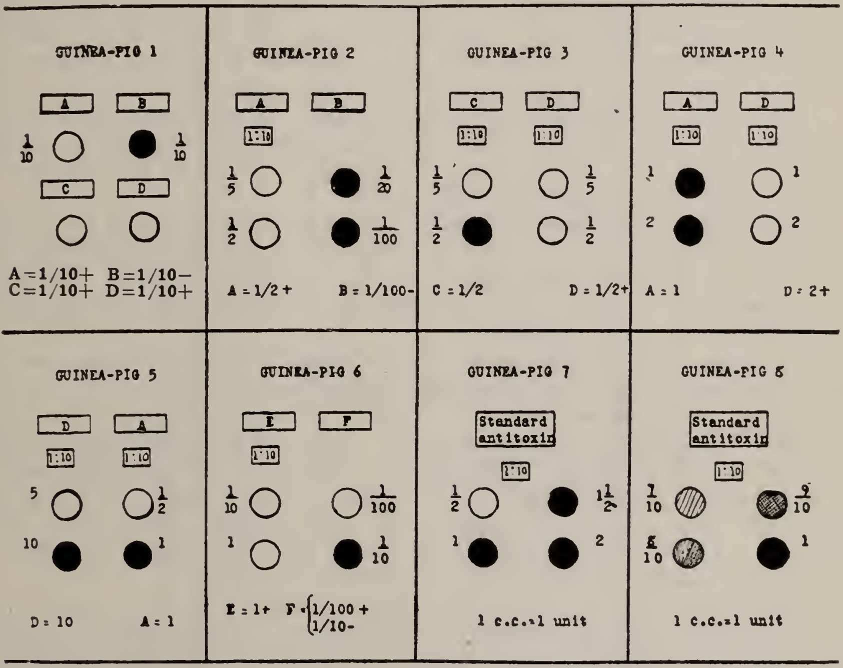

TABLE III.

SAMPLE PAGE OF DAILY RECORD OF TESTS.

Sera.	Guinea-pig.	Place of Injection.	Amount of Antitoxin Tested For.	Daily Observations.				Result of Tests.
				Feb. 13.	Feb. 14.	Feb. 15.	Feb. 16.	
A	1	R U	1/10 unit	OK	OK	OK	OK	1/10 +
B	1	R L	1/10 unit	Sl. r	Mod. R	Id. n. R	Id. N. R	1/10 —
C	1	L U	1/10 unit	OK	OK	OK	OK	1/10 +
C	1	L L	1/5 unit	Sl. r	Mod. R	Mod. n. R	Mod. n. R	1/5

R U, R L, L U, L L refer to the right upper, right lower, left upper, and left lower quadrants, respectively, of the guinea-pig abdomen.

OK = no reaction; Sl = slight induration; Mod = moderate induration; Id = marked induration. N = marked necrosis; n = slight necrosis. R = marked redness; r = slight redness.

Guinea pig 1 (Table 2) shows the preliminary results of tests on 4 different sera for 1/10 unit of antitoxin. Sera, A, C and D completely neutralized the toxin solution and no lesions appeared. Serum B gave a necrosis. Results: A, C and D had more than 1/10 unit, B less than 1/10 unit of antitoxin to the cubic centimeter.

Guinea pigs 2 and 3 represent the 2nd set of tests on the 4 sera. Serum A showed no lesion with the ½ unit test, therefore more than ½ unit was present. Serum B showed less than 1/100 unit, a necrosis appearing with this test. The serum can be further tested for 1/200 unit and if a definite lesion appears the serum is considered as having no antitoxin. Serum C showed no lesion with the test for 1/5 unit, but a slight lesion with the ½ unit test. Antitoxin content therefore was ½ unit to the cubic centimeter. If a marked lesion had developed, an intermediate test for 1/3 unit could be made. Serum D had more than ½ unit.

Guinea pig 4 represents the 3rd set of tests for Sera A and D. Serum A showed a lesion with tests for 1 and 2 units. The antitoxin content was therefore 1 unit, since the test in Guinea-pig 2 for ½ unit showed no lesion. Serum D showed more than 2 units.

Guinea pig 5 represents the 4th set of tests for Sera A and D. Serum D showed a lesion with the test for 10 units and no lesion with the test for 5 units. The antitoxin content therefore equaled 10 units. Serum A was retested for ½ and 1 unit to show the non-necrosing and necrosing test points on the same animal.

By using 5 animals we were able to make 20 different tests, and arrive accurately at the antitoxin content of 4 different sera.

Guinea pig 6 shows 2 sera, E and F, on which the tests were made for widely separated antitoxin contents. This can be done when we wish to arrive rapidly at rough limits of the amount of antitoxin in a serum. Serum E showed more than 1 unit, Serum F more than 1/100, but less than 1/10 unit. Serum E would have to be tested further for 10,100 units, etc., while Serum F should be tested for 1/20 and 1/50 units, if we wished to establish the accurate amount of antitoxin.

Guinea pig 7 shows tests made by this method on the standard antitoxin furnished from the Hygienic Laboratory in Washington, of which each cubic centimeter represents exactly one unit of antitoxin. With the intradermal test the antitoxin content was likewise definitely established as 1 unit to the cubic centimeter, since no lesion appeared with the test for ½ unit, but a distinct lesion with slight necrosis in the test for 1 unit. The test for 1½ and for 2 units showed more marked necrosing lesions.

Guinea pig 8 shows the same standard antitoxin tested for 0.7, 0.8, 0.9 and 1 unit. Slight inflammatory lesions were noticed even with the test for 0.7 unit. It is therefore best not to attempt to make too fine a differenctiation between the slight lesions of closely lying tests, but to endeavor to obtain the necrosing and non-necrosing points of a serum, the first representing twice the amount of antitoxin tested for in the second. When we are dealing with concentrated antitoxin sera (100-1000 units to the cubic centimeter) the intradermal test can give us only a preliminary idea of the antitoxin content; the more accurate estimation is subsequently made by the subcutaneous test.

This method is applicable not only for determining the antitoxin content of sera from human beings, but also from animals. The following table is a specimen test page from the animal record book showing the results of successive tests for antitoxin in the blood of horses before and after these animals have been injected with toxin-antitoxin.

TABLE IV.

TESTS ON HORSES INJECTED WITH TOXIN-ANTITOXIN.

TESTS FOR ANTITOXIN IN CONTROL BLEEDINGS TAKEN BEFORE THE INJECTION OF THE TOXIN-ANTITOXIN, AND ON TWO TEST BLEEDINGS TAKEN AFTER THE INJECTION.

Horse Injected.	Injections of Toxin-Antitoxin "M."			Control Bleeding Dec. 31.			Test Bleeding 1 Jan. 14.			Test Bleeding 2 Jan. 21.			Results of Tests.		
													Control.	Test 1.	Test 2.
644	Jan. 2 0.25 cc.			Jan. 27 0.1+	Jan. 30 0.2		Feb. 2 0.5+	Feb. 5 2+	Feb. 8 4	Feb. 10 4+	Feb. 13 8—	Feb. 16 6	0.2	4	6
645	Jan. 2 0.25 cc.	Jan. 5 0.25 cc.	Jan. 8 0.25 cc.	Jan. 27 0.1—	Jan. 30 0.02	Feb. 3 0.01+	Feb. 5 0.1+	Feb. 8 0.5+	Feb. 12 2	Feb. 15 4+	Feb. 18 8		0.02	2	8
648	Jan. 2 1 cc.			Jan. 27 0.1—	Jan. 30 0.01—	Feb. 3 0.005—	Feb. 8 0.1—	Feb. 12 0.05	Feb. 15 0.02+	Feb. 15 0.05+	Feb. 18 0.1		0.005—	0.05	0.1
651	Jan. 2 2 cc.	Jan. 5 2 cc.	Jan. 8 2 cc.	Jan. 27 0.2+	Jan. 30 0.5		Feb. 5 1+	Feb. 8 4+	Feb. 12 8	Feb. 15 8	Feb. 18 16—	Feb. 22 4+	0.5	8	8

CONCLUSIONS.

An intradermal test is presented for estimating in an economical way small amounts of antitoxin.

The method is easily carried out, but is requires for accuracy a uniform technic in making the dilutions of toxin and serums, the allowance of a uniform time period for the two to combine, uniformity in the method of injection into the guinea pig, daily observations of the animals, and uniform interpretation of the tests.

Problems in experimental work in diphtheria and the accurate serologic control of actively immunized individuals can be carried out by this test.

ON THE VALUE OF THE PETROLEUM-ETHER METHOD FOR THE ISOLATION OF B. TYPHOSUS FROM FECES.*

Lawrence A. Kohn and Charles Krumwiede, Jr.

The use of petroleum-ether in the isolation of typhoid bacilli from feces was suggested by Bierast (1914). He added a finger's breadth of petroleum to a fecal suspension in broth and shook the mixture at intervals. After allowing this to stand in a cool place for sixteen hours, he plated the sediment or some of the broth from the bottom of the container. He reported that 2 out of 23 specimens were positive only by this method. The work was interrupted because he developed typhoid fever. Jaffe (1915) had favorable results with 24 specimens, as follows:

Number.	Direct Plating.	After Shaking, Etc.
5	—	—
5	+	+
0	+	—
4	—	+

He calls attention to the danger of the method and reports a case of typhoid fever due to its use.

Hall (1915) working with the method found that the activity of the different fractions obtained by the distillation of crude petroleum varied inversely with the boiling point. He found that the fraction with a boiling point about 40 deg. C. gave the best results. This fraction would contain a large proportion of pentane (C_5H_{12}) and for convenience we shall refer to it as such.

Hall modified the Bierast procedure as follows. To a moderately heavily suspension of feces in broth is added one-half its volume of pentane. Close the bottle tightly with a rubber stopper and shake for one-half hour in a shaking machine. The container is then allowed to stand for one and one-half to two hours at room temperature and the bottom fluid or sediment removed with a pipette and plated. His results may be summarized as follows:

Specimens Examined.	Direct Plating.	Pentane Shaking.
69	Positive, 13	Positive, 13
	Positive, 1	Negative, 1
	Negative, 7	Positive, 7
	Negative, 48	Negative, 48

Thus, while one specimen positive on direct plating was negative after shaking, 7, or 33 per cent of the total positives, were positive only after shaking.

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These results were so favorable that we investigated the method. Unless otherwise stated, we have followed the method of Hall.

Our first attempts were made with stools from typhoid carriers and with greatly diluted broth cultures of recently isolated typhoid strains. The shaking was for one-half hour, and after shaking, the emulsions were allowed to stand for two hours before plating on the Endo medium. The results may be roughly illustrated by the following examples:

Material.	Direct Plating.	After Shaking.
Stool suspension	Positive—5 per cent. typhoid	1 typhoid colony
Stool suspension	Positive 4,000 total colonies; 75 per cent. typhoid	100 total colonies; 90 per cent. typhoid
Stool suspension	Positive 5,000 total colonies; 5 per cent. typhoid	200 total colonies; 1 per cent. typhoid

Broth suspensions of several cultures (pure), yielding by direct plating from 400 to 5000 colonies per plate, gave no colonies after shaking and then plating an equivalent amount of material.

While, with the one stool cited above, there was a relative increase in the proportion of typhoid to total flora, it would obviously be dangerous to expose stools less rich in typhoid to the risk of this marked absolute decrease in typhoid colonies. We next tried shortening the time of shaking, and found that in no case did the proportion of typhoid increase on shaking longer than ten minutes, whereas the total number recovered frequently took an alarming drop, and in some cases none was recovered after thirty minutes. It was also determined at this time, that more typhoid bacilli were viable after one than after two hours' standing. The results with this modified technique—that is time of shaking reduced to ten minutes and time of standing to one hour—may be summarized briefly as follows:

Total Examined.	Direct Plating.	After Shaking.
12	Positive, 10	Positive, 10
	Positive, 1	Negative, 1
	Negative, 1	Positive, 1

These stools were quite rich in typhoid. Of those in which bacilli were found both before and after shaking, the percentage of typhoid to total flora was increased in three and decreased in seven cases. In 3 of the 7, less than 10 per cent of the number of colonies on the direct plates developed after shaking. In the case of the 3 specimens showing relative increase, 2 of the 3 specimens showed loss of typhoid bacilli, reduced in one case to 3 per cent of the original number. The stool which gave positive results only on direct plating had 75 per cent of typhoid (total colonies 900 and yielded 150 non-typhoid colonies after shaking. That in which typhoid bacilli were found only after shaking had 6000 colonies on two badly diffused direct plates, which were reduced on shaking to 400, of which 20 were typhoid. In other words, the tendency, even with the shortening of the time of exposure, is to a rapid reduction of the number of typhoid bacilli; but this is not associated necessarily with a relative increase as compared with the accompanying flora.

While these experiments were under way, we conducted a number of tests with pure cultures of recently isolated strains of typhoid, designed to develop a technique more favorable than that which we were employing. First, we determined that an emulsion of typhoid in broth was not more susceptible to the action of pentane than a similar emulsion in either a normal stool suspension, or in the same suspension sterilized by steaming for one-half hour. Thus:

Summary of reactions of several strains: shaken ten minutes, stood one hour

Direct plating	*After shaking*
In broth 2500 colonies	0
In stool suspension 2500 colonies, 95 per cent. typhoid	0
In sterilized stool suspension 2500	0

On attempting to find the approximate percentage of typhoid bacilli which would survive shaking alone, we found wide variations between individual strains. With some, 250 colonies per drop were completely lost on shaking; with others, the numerical reduction varied from 40 to 98 per cent. With this reduction in numbers, there was frequently a concomitant reduction in the size of the colonies developing after shaking. It was thought that the physical effect of shaking might be responsible, but on shaking without pentane (with equivalent saline dilution) practically no reduction occurred, whereas, 5000 colonies per loop were killed by pentane in material from this case. It was thought that the bacilli might be contained within the supernatant pentane rather than in the subjacent broth, but cultures from the pentane in two cases where the broth yielded from 3 to 25 colonies per drop, showed it to be sterile.

We then investigated more accurately the effect of standing after shaking. The results with several strains were as follows:

Direct Plating.	Immediately After Shaking.	Stood 15 Minutes.	Stood 30 Minutes.	Stood 45 Minutes.	Stood 1 Hour.
5,000*	3,000	3,000	2,000	2,000	1,800
3,000	600	160	65	27	25
1,400	400	21	12	12	3

*Strain (in other tests) shows unusual resistance to pentane.

In a final series of stool examinations, small amounts of carrier stool were added to normal stools so as to give mixtures poor in typhoid. Obviously, it is only with this class of material that the method, if of value, will aid in diagnosis. In order to determine more accurately the initial number of typhoid bacilli, we incorporated the use of the brilliant green medium described by Krumwiede, Pratt and McWilliams (1916), which has proved extremely successful in the isolation of typhoid bacilli from feces. As preliminary tests had shown that in stools as well as in broth cultures, the typhoid bacilli were more or less rapidly lost on standing after the shaking was over, we plated immediately after shaking ten minutes with pentane, and obtained the following results.

Total Examined.	Direct Endo.	Direct Green-Dye.	Endo After Shaking.
50	Positive, 21	Positive, 21	Positive, 21
	Negative, 7	Positive, 7	Positive, 7
	Negative, 9	Positive, 9	Negative, 9
	Negative, 13	Negative, 13	Negative, 13
Total positives	21	37	28
Percentage positive	42.0 per cent.	74.0 per cent.	56.0 per cent.
Percentage positive compared with total number positive	56.7 per cent.	100.0 per cent.	75.6 per cent.

These results indicate that the method with very short exposure has some value as compared with the direct plating on a non-restraining medium (Endo) alone. The general tendency to reduction in the number of typhoid bacilli, not necessarily associated with a markedly greater decrease in the fecal flora, was again observed in this series. On the whole, the data presented indicate that the method is not a reliable one for the relative enrichment of typhoid bacilli in stools. Compared with direct plating on a differential medium (brilliant green agar) the method is evidently inferior.

A report by Schuscha (1916) recently available only in an abstract indicates that his results with the method have not been more satisfactory than with direct plating.

We might add that the method of pentane shaking is unsuited to routine laboratory work. The pentane itself is highly inflammable, and the containers are protected from leaking during agitation in the machine only with difficulty. Two workers abroad have developed typhoid fever while using the method and we feel that there is a large element of danger in its use because of the tendency for the contents of the bottles to blow out when opened, due to the highly volatile pentane.

CONCLUSIONS

The petroleum-ether method for the detection of *B. typhosus* has no advantage over direct plating on a medium which restrains the growth of the associated fecal types. With short periods of exposure, it may be successful where direct plating on Endo alone fails. There is a strong tendency to reduction of the number of *B. typhosus,* not necessarily associated with a greater reduction of the accompanying fecal bacteria. Moreover the danger of infection to those using the method is sufficiently serious to warrant its condemnation, especially as it offers no advantage over direct plating on differential restraining media.

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STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

I. Xylose Fermentation for the Differentiation of B. Paratyphosus "A" from Other Members of the Paratyphoid-Enteritidis Group.*

Charles Krumwiede, Jr., Josephine S. Pratt, and Lawrence A. Kohn.

Over a year ago we gave a preliminary report[1] on the differentiation of the members of the paratyphoid-enteritidis group. Although we have been unable to complete this work, one fermentation reaction seems to us of sufficient practical importance to warrant a report without further delay.

As far as we know, no one, with the possible exception of Harding and Ostenberg, has suggested a method based on a carbohydrate fermentation for the differentiation of the Paratyphoid A types from other paratyphoid-enteritidis types. We have found that the A types which we have, uniformly fail to ferment xylose, whereas the other types do. The fermentation, where positive, usually occurs promptly (eighteen hours) which is of practical importance. Only one strain fermented slowly but even in this case a slight change of color of the indicator was noticeable within the time given. This strain, a so-called "hog-cholera," is atypical in other respects and may prove not to be a true paratyphoid type on further study.

The cultures studied are as follows:

Paratyphoid A	20	strains
Paratyphoid B	15	"
Typhi murium	7	"
Guinea-pig, rabbit, cat, etc.	13	"
Hog-cholera	24	"
B. enteritidis	5	"
B. danysz	4	"
B. abortus equinus	12	"
Total	100	strains.

All the xylose-negative types have been tested agglutinatively. Two groups were found; one was agglutinated only by "A" type serum, the other (five strains) was not agglutinated by either "A" or "B" type sera. (That is, in dilutions beyond the range of group and normal agglutinins.) Only a moderate number of the xylose-positive types have been identified agglutinatively, but in no instance was there a discrepancy between the xylose result and agglutination.

The fact that not all strains culturally similar to the paratyphoid A group are agglutinated by A serum has been noted by others. Thus Bainbridge[2] speaks of a strain isolated in India, culturally identical with B. paratyphosus A, but not agglutinated by paratyphoid (A) serum. His warning, however, against including in this group types not agglutinated by "A" type serum, unless there is sufficient evidence that they are the actual causative agent in the disease process, is not amiss. Paratyphoid-like intermediate types are constantly being encountered, a fact not sufficiently appreciated by many workers. The non-agglutinating strains which we have were received as pathogenic types, isolated from human disease. It may be that further work will reveal some cultural variations in these strains. We hope to report later on the inter-agglutination relationships of the xylose-negative group and to include further cultures in this study.

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(As strains of B. paratyphosus "A" are difficult to obtain, we should be very grateful to any one sending us recently isolated cultures).

Uhlenhuth and Hübener[3] cite a series of cases from which bacilli belonging to the paratyphoid group were isolated. These were not agglutinated by sera of the fixed types. They suggest that strains of this character be included in a paratyphoid "C" group. More recently, Meyer has suggested that B. abortus equinus be included in this group. The nomenclature is unsatisfactory and we would suggest, tentatively, that the types of human origin which fail to ferment xylose be included in the paratyphoid "A" group and that sub-groups be made according to agglutinative differences. This would have the advantage of considering host-origin in the classification.

Xylose has been used by many workers. Uhlenhuth and Hübener[4] give xylose fermentation as a characteristic of B. paratyphosus and B. enteritidis, as well as for the various types of animal origin included under these names. Harding and Ostenberg,[5] already mentioned, found in a small series of cultures that there was a difference in xylose fermentation, and suggested the differential value of this carbohydrate. They do not seem to have appreciated its possible value in separating the "A" from the "B" types. They include a "typhi murium" strain among the xylose-negative group, but apparently did not study this strain to determine its real identity. A true typhi murium type should ferment this carbohydrate, according to the results of all other workers, as well as our own. We obtained what is supposed to be the same strain they used, and it fermented xylose. We have found that rodent strains apparently have a lower avidity for xylose than other paratyphoid-enteritidis types. Whether this is found to be sufficiently constant for differential purposes will depend on work now in progress. A xylose-negative group, therefore, among strains of animal origin, is a possibility, but one should be careful in identifying such strains, as paratyphoid-like intermediate types are commonly encountered, and not a few stock strains belong to the latter category. Even should such strains be found, it would not detract from the value of xylose in the differentiation of strains of human origin.

The medium used has varied, but a peptone (Witte's) water, containing peptone one per cent. and salt .5 per cent. with one per cent. of xylose (Kahlbaum's), and one per cent of the Andrade indicator, has given most satisfactory results. Slight adjustment of reaction may be required. The usual precautions against overheating the medium after the carbohydrate is added are indicated. As the carbohydrate is relatively expensive, we have used 1.5 cubic centimeters of the medium in narrow test-tubes.

Summary.—In a series of cultures representing nearly all the pathogenic types of the paratyphoid-enteritidis group, a group of cultures, including all the types agglutinatively B. paratyphosus "A," failed to ferment xylose. We suggest, if the study of further strains shows this to be a constant characteristic, that the xylose-negative types from man be considered the paratyphoid A group on cultural grounds. Within this cultural group are encountered strains, presumably pathogenic, which differ agglutinatively from the normal "A" type.

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STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

II. Observations on the Reaction in Litmus Milk as a Method of Biological Differentiation.*

Charles Krumwiede, Jr., Josephine S. Pratt, and Lawrence A. Kohn.

Milk, with or without litmus, or litmus whey, is generally accepted as a medium of differential value in separating B. paratyphosus "A" from other members of the paratyphoid-enteritidis group. The following gives the accepted differential characteristics:

Medium.	B. Paratyphosus A.	B. Paratyphosus B, B. Enteriditis, and Similar Types.
Milk..........	No visible change, acid reaction.	At first unchanged, reaction acid. After 1 to 3 weeks, yellowish, transparent and alkaline.
Litmus milk...	Acid, continued; no change to blue.	Usually an initial acidity; then alkaline, usually after several days or longer.
Litmus whey...	Acid, continued; no change to blue.	Usually an initial acidity; alkaline, usually after several days or longer.

Plain milk has not obtained a general acceptance as the changes are not evident as quickly as with the other media. Litmus whey has been used nearly exclusively by the Continental workers and is given as the preparation of choice by Uhlenluth and Hübener.[1] Because of its greater simplicity, litmus milk has been widely used and all the reports indicate that litmus milk and litmus whey give identical results.

We were led to make a study of this reaction because of the atypical results obtained with two strains which were subsequently found not to be true to name. Other strains were tried and as the results did not agree with those usually described, a larger series of cultures was studied.

The litmus milk employed was made from a "certified" milk of low bacterial count. The milk in bulk was steamed in the Arnold sterilizer for one-half hour, and then placed in the refrigerator to allow the cream to rise. The milk was then separated by syphon, 5 per cent. of Kubel and Tiemann's litmus solution added, tubed, and sterilized in the Arnold by heating for twenty minutes on three successive days.

The inoculated milk tubes were observed at the end of twenty-four hours, and then usually every other day until a blue color was noted. After fourteen days, weekly observations only were made.

Analyzing the results as to the time when the inoculated milk became distinctly bluer than the control, gives the following:

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* Published in Jour. Med. Res., 1916, 35, p. 55.

Type of Strain.	Number of Strains.	Time of Appearance of Blue Color.	Notes.
B. paratyphosus "A".....	3	....	Reached color of control after 2 months.
	6	5-6 weeks.	
	6	3-4 "	All violet at 48 hours with one exception; this was the color of the control and was blue on the fifth day.
	2	2 weeks	
	2	7 days.	
	3	5 "	
B. paratyphosus "B" including................	12	5 days.	2 violet at 48 hours.
	7	4 "	17 like control at 48 hours (compare also next table).
	3	2 "	
Hog cholera types....	1	5 days.	Violet at 48 hours.
	4	4 "	Like control at 48 hours. 15 other strains only observed after one week; were blue.
Typhi Murium* types.	1	14 days.	Like control at one week.
	1	7 "	Violet on 5th day.
	1	5 "	Violet on 2d day.
	2	4 "	Like control on 2d day. 5 other strains only observed after one week; were blue.
Guinea-pig types.....	1	5 days.	Violet on 2d day.
	3	4 "	Like control on 2d day.
B. enteritidis including....	3	5 days.	Like control, 2d day.
	2	2 "	
Danysz types........	1	4 days.	Like control, 2d day.
	2	2 "	
B. abortus equinus........	1	5 days.	Like control, 2d day.
	1	2 "	

*These types are tentatively placed in the "B" group. As we have not identified them agglutinatively possibly some may belong in the enteritidis group. Of the two typhi murium strains, the one becoming blue only by the fourteenth day belongs, agglutinatively, to the Paratyphoid "B" group. The other is agglutinated neither by Paratyphoid B nor by Enteritidis sera.

The table shows a wide gradation in rapidity in reaching the blue stage among the "A" types. Those "A" types becoming blue more rapidly merge imperceptibly with the other types which as a group become blue more quickly. Not included in the above table are two strains obtained in triplicate from different institutions. These, as well as sub-strains isolated by plating several of the cultures, gave such wide variations that they are tabulated separately. They belong to the "B" group agglutinatively and in all other cultural reactions.

OBSERVATIONS ON IRREGULAR STRAINS.

	2d Day.	6th Day.	14th Day.	21st Day.
S 1***	Violet.	Blue.	—	
" 2	"	"	—	
" 3	"	"	—	
" (65)	"	Violet.	Violet.	Bluish.**
" (122)	"	Control.*	Blue.	—
M 1	"	Blue.	—	—
" 2	"	"	—	—
" 3	"	Violet.	Blue.	—
" (66) 1	"	"	Violet.	Bluish.**
" (66) 2	"	"	"	"
" (66) 3	"	"	"	"
" (121)	"	Control.	Bluish.**	Blue.

*Slightly bluer between 6th and 14th day.
**Bluish=very slightly more blue than control.
***1–2–3=separate isolations from original cultures.

The variations are of interest when compared with the preceding table showing the gradient character of the reaction. These variant strains increase the number and degree of intermediate reactions.

A moderate number of other duplicate cultures were tried with slight variations in most instances. Two "A" type cultures, presumably the same strain, showed variations on one test. One became bluer than the control by the fifth day, the other returned to the color of the control by the seventh day and did not definitely shade to blue until the third week.

It may be objected that although the "A" types became bluer than the controls, the degree of alkalinity was less than that produced by the other types. This was true but at any period of observation up to two weeks, at least, intermediate grades of color were given by some strains of both types so that a cleavage was difficult; thus neither the factor of intensity of color nor the factor of time could be used as a basis for division. As a matter of fact, some of these gradations are not brought out in tabulating the results.

As an example of this gradation the following actual observations will suffice. Although the individual strains fluctuated in position on different observations, the types only are stated, as the results are thus more easily interpreted.

Litmus Milk, inoculated 10/21/14:

Observations (some of the duplicates mentioned above are included):

10/22: All more violet than control, slight variations in intensity of color.

10/23: Arranged according to gradations of color, the differences are slight, so that grouping is not sharp; the shades of color grade as follows:

Bluer than control, fading in the order given,—Ent., B, B, B, Ent.

Like control, but showing slight shading from blue to violet tinge in the order given,—B, Ent., B, Ent., B, Ent., B, B, B, B, B, A, B, A, B.

More violet than control; slight intensification in order given,—B, B', B, A, B, B, A, B, B, A, A, B, A, A, B, A, A.

Observations, 10/27: Bluer than control, but approaching color of control in the order given,—Ent., B, B, B, B, Ent., B, B, B', B, Ent., B, B, B, Ent., Ent., B, B, B, B, B, B, A, B, B', A, B, A.

Like control shading into violet in order given,—A, A, A, A.

More violet than control,—A, B, B, A, A.

Observations, 11/5: Definitely deep blue,—Ent., B, B, B, B, Ent., B', B, B, B, B,

Ent., B, B, B, Ent., Ent., B, B B.
Shading to lighter blue, all bluer than control,—B, B, B, B, A, B, A, A.
Bluish violet, deeper than control—A.
Violet,—A, A, A, B, B, A, A, A.

Later observations: By the twenty-first day all of the remaining "B" types became bluer than the control as well as some of the 'A" types, giving the general results already tabulated.

The results show that according to the reaction in litmus milk the paratyphoid-enteritidis group falls into two general sub-groups. The first group, representing most of the "A" types, produces an initial acidity which persists for a relatively long time. The second group, representing the "B" and enteritidis types as well as the strains usually included under these terms, and B. abortus equinus, usually produces an initial acidity followed by prompt alkali production. Gradations according to time and degree are present in both groups. A moderate number of "A" and "B" types give intermediate reactions, thus causing the two groups to merge.

Although these results are essentially at variance with the usually accepted views, we find that others have had aberrant results, although not as extreme as ours. Uhlenhuth and Hübener [2] give litmus whey as a reliable differential medium, but quote variations that have been noticed. Most of these were apparently caused by an unsatisfactory whey, but some variations have been due to the strains. In those instances, alkalinity has developed slowly or the return of color has not gone beyond that of the control tube. Boycott [3] noticed that two "A" types produced a blue color in litmus milk after two months and suggested that the differences in this medium might be only of temporal degree. Bainbridge [4] says that the "A" types as a rule produce permanent acidity after the lapse of several weeks. Buxton,[5] among the earlier workers, noted that one strain was erratic, at times promptly turning the milk blue, at times leaving the milk acid for a considerable time. His titrations are of interest as, even where the litmus showed no alkalinity, the titration with phenolphthalein showed less acidity than the control tubes, indicating some alkali production.

We thought that possibly the substitution of the Andrade indicator (100 cc. of 0.5 per cent. aqueous solution of acid fuchsin decolorized by the addition of 16 cc. of normal NaOH solution) might give sharper results. One per cent. of the indicator was used. This indicator is much more delicate than litmus. Media containing this indicator are pink when acid and colorless when alkaline. We counted also on reduction, given as an essential property of the members of the paratyphoid-enteritidis group other than the A types. That is, after the initial acid pink, reduction of the dye would occur and the change to a colorless state, which would normally develop from subsequent alkali production, would be accelerated.

In general sharper results were obtained with this indicator; but the same general tendency to gradation persisted. In this respect the results control those obtained with litmus milk. The aberrant "B" types, however, gave similar results. The following gives briefly the results with the strains tried. Atlhough the cleavage is sharper than with litmus milk, these strains are so erratic that they rob the reaction of its differential value.

Type.	Number of Strains.	Time when Less Color than in Control.
B. Paratyphosus A	5	30 days.
		10-14 days.*
	3	5†-7 days.
Other types	50	2-4 days.

*Some strains omitted, observed only to twenty-first day when they were the color of the control.
†Two of the strains were only slightly lighter than the control on the fifth day.

IRREGULAR STRAINS.

Symbol	1	S 2	S 3	S (65)	S (122)	M 1	M 2	M 3	M (66)$_1$	M (66)$_2$	M (66)$_3$	M 121
Time in days when color less than control	12	7	6	6	1	9	4	10	6	12	12	7

In tabulating the results, however, the gradations are somewhat broken, as was the case with litmus milk. To show this one observation will suffice, thus:

Milk (Andrade indicator) inoculated 10/21/14:

Observation 10/26: Deeper than control (more acid)—deep pink to pale pink in order given,—B,* B,* A. A, A, A, B.* B,* B,* B.* A, A, A, A.

Like control to very slightly lighter than control,—B,* B,* A, A, B.*

Paler than control, most of those toward the right nearly colorless to colorless,—B.* B, Ent., B, Ent., Ent., B, B, Ent., B, B, B, B, B, B, B, B, B, B, Ent., B.

*Irregular strains and separate isolations.

Conclusions.—With the strains we have studied, the reaction in litmus milk of the different members of the paratyphoid-enteritidis group is a gradient one. Although most of the paratyphoid "A" types produce alkali more slowly than the other members of the group, this difference is quantitative only and intermediate degrees of reaction, both temporal and quantitative, destroy largely the differential value of the medium for these organisms. The usually described qualitative reaction, therefore, has not been verified by our results. With milk containing Andrade indicator, somewhat sharper differences are obtained with most of the strains, although the general quantitative character of the reaction is preserved. The somewhat sharper differences are due probably to the greater delicacy of this indicator and coincident reduction of the color. Some strains, however, react irregularly on this medium, as on litmus milk, and show still further its lack of value when used as a qualitative method of differentiation.

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STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

IV. The Differentiation of the Members of the Paratyphoid-Enteritidis Group from B. Typhosus with Special Reference to Anaerogenic Strains and Observations on the Fermentative Characteristics of the Avian Types.*

Charles Krumwiede, Jr., and Lawrence A. Kohn.

Although the majority of the members of the paratyphoid-enteritidis group are readily differentiated from the typhoid bacillus by their ability to produce gas from glucose, the isolation of an increasing number of strains (Moore,[1] Dorset,[2] Bock,[3] Oette,[4] Wagner,[5] Ohno[6]) which, though failing to produce gas, must be included in this group, makes necessary a basic biological group characteristic that will separate anaerogenic as well as aerogenic types from B. typhosus. Ten Broeck [7] and others have also shown that the ability to produce gas from glucose may vary or even be suppressed. Of particular interest is the anaerogenic B. sanguinarium (Fowl Typhoid, Moore). Smith and Ten Broeck [8] have shown that this organism has a very close cultural and immunological relationship to B. typhosus, although differing in its lack of motility and further separable by agglutinin absorption. They also showed that B. pullorum (Rettger), although producing gas normally from glucose, is also closely related immunologically to B. typhosus and to B. sanguinarium as well.

In our study of the aerogenic members of the paratyphoid-enteritidis group we found that rhamnose was uniformly and promptly fermented, whereas the typhoid bacillus did not attack this carbohydrate. These observations confirm the results of previous workers, who, as a rule, however, seem to have attributed no special practical importance to these findings. Thus, Uhlenhuth and Hubener [9] in their summary give the fermentation of rhamnose as a characteristic of the paratyphoid-enteritidis group, but state that the fermentation (gas production) of glucose alone suffices to separate this group with certainty from B. typhosus. Oette,[4] however, in studying the non-gas-producing "B" type he isolated, showed that it was related culturally to B. paratyphosus and not to B. typhosus, because of its ability to ferment rhamnose. Wagner,[5] in a study of two strains, made the same observation. Both these authors give data indicating that gas production by these organisms is subject to variation in the human body. We have extended the study of rhamnose fermentation to the available anaerogenic types, especially B. sanguinarium, and to the aerogenic B. pullorum. Other carbohydrates were included, primarily to elicit, if possible, further differences among the three closely related types: B. typhosus, B. sanguinarium, and B. pullorum.

The medium employed was a peptone water containing one per cent of Witte's peptone, 0.5 per cent of salt, one per cent of the Andrade indicator, and one per cent of the carbohydrate. Unless otherwise stated, acid production alone was observed. The fermentative results are given in Table I.

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TABLE I.

	Gas from	Acid Production (Number of Strains Studied and Day of Evident Fermentation).								
	Glucose.	Mannite.	Maltose.	Xylose.	Sorbite.	Dulcite.	Arabinose.	Rhamnose.	Inosite.	Adonite.
B. typhosus (37 strains)	—	+	+	29+ 1+ 5 to 9 5+ 8 to 10 2+11 to 13	36+ 1+2	2+ 5 to 7 6+ 8 to 10 16+15 to 21 5—	34— 2†—or+	—	—	—
B. sanguinarium No. 180	—	+	+	+1 to 2	(See notes.) +9	+1 to 2	+	+2 to 5	—	—
B. sanguinarium No. 203					—14 | + 9					
B. sanguinarium No. 204					+11 | +14					
B. sanguinarium No. 205					—14 | —31					
B. sanguinarium No. 206					+ 8 | +14					
B. pullorum (13 strains)	+	+	—14*	+1 to 2	+2 to 7	—	+	+	—	—
B. suis (Dorset strain)	—	+	+	+	+	+or—**	—	+	—	—
B. suis No. 152	—	+	+	+	+2	+or—**	—	+	—	—
B. suis (Ten Broeck strains)	—	+	+	+	+	+or—**	—	+	—	—
“ “ “ “ “	+	+	+	+	+	+	—	+	—	—
B. paratyphosus No. 93	—	+	+	+	+	+	—	+	—	—
Control Strains. B. paratyphosus “A”	+	+	+	—	+	+	+	+	—	—
Control Strains. “ “ “B”	+	+	+	+	+	+	+	+	+	—
Control Strains. B. enteritidis	+	+	+	+	+	+	+	+	—	—

Explanation of Symbols: + = evidence of fermentation within 24 hours. Figure following + is interval in days before positive fermentation was observed Figure preceding + refers to number of strains. Negative results (—) mean observation for 21 days unless otherwise noted.

Notes: †These two strains (recent isolations) discussed below. *Later observations given in text. **Irregular, negative, or delayed positive results Sorbite: duplicate observations with B. sanguinarium.

Source of Cultures: B. sanguinarium No. 180 is Smith and Ten Broeck II. c. We are indebted to Dr. K. F. Meyer for the other four strains. The B. pullorum strains were obtained from Drs. Smith, Rettger, and Meyer. The strains B. suis No. 152 and B. paratyphosus “B” No. 93 are stock strains, the origin of which is not ascertainable. The strains of B. typhosus are, with four exceptions, recently isolated fecal strains.

The time of appearance of acid production is noted, as we found that differences in avidity as well as variability due to the enhancement of low or latent avidity were significant in correlating the relationships of the various groups and of practical importance in their separation. This is best brought out by discussing the more essential carbohydrates employed.

Xylose is fermented by all members of the paratyphoid-enteritidis group with the exception of B. paratyphosus "A." B. typhosus is more closely related to the latter and this correlates with the fluctuating avidity shown by different typhoid strains for this carbohydrate. It is suggestive that B. sanguinarium and B. pullorum, closely related to B. typhosus, do not show a maximum avidity.

Dulcite is slowly fermented by the majority of strains of B. typhosus, although it is usually stated that this carbohydrate is not so fermented. Penfold[10] has noted this ability to ferment dulcite slowly, and by cultivation on dulcite media obtained strains which fermented promptly. As has been noted by many observers, there is a gradient avidity for dulcite in the paratyphoid-enteritidis group, B. paratyphosus "A" attacking the carbohydrate slowly, B. paratyphosus "B," and B. enteritidis fermenting more promptly, the latter in general showing the greatest avidity. The intermediate avian strain B. pullorum, and some B. suis strains, break the gradation from B. typhosus to B. enteritidis. B. sanguinarium and B. pullorum differ in relation to this carbohydrate. The anaerogenic B. suis types show a fluctuating avidity for dulcite. It is interesting to note the differences between the two Ten Broeck[7] strains in this respect; apparently the loss of gas production is associated with a partial suppression of the ability to ferment dulcite.

Arabinose was fermented in some tests by two strains of B. typhosus, T. 9 and T. 38. Both show a further peculiarity in that they are unusually sensitive to the action of brilliant green. Further observations have shown that this latent ability to ferment arabinose can be considerably enhanced. The stock culture of T. 9, which gave a positive result in seven days in one test and remained negative for four weeks in two other tests, was plated and six fishings isolated. One of these fermented in three days, the other five were negative after three weeks. The positive fermentation tube of the original culture was similarly plated, and of six fishings four were positive in twenty-four hours, one in nine days, and the other remained negative after twenty-six days. Evidently growth in the arabinose medium was followed by adaptation, or more probably by selective multiplication of the individuals more capable of fermenting this carbohydrate. All the fractions were typical agglutinatively. The other strain, T. 38, showed a similar adaptation, but the positive results were much slower in developing. Similar results have been noted by Penfold.[11] He found that strains of B. typhosus fermented arabinose slowly and irregularly, but by sub-culture in arabinose media a promptly and regularly fermenting strain could be produced. Some strains of typhoid, therefore, normally possess the ability to ferment arabinose, but their avidity for the sugar is low and appears irregularly. Other strains probably all possess a latent ability which could be developed by appropriate means. From the practical standpoint, however, this does not detract from the differential value of this carbohydrate in separating B. sanguinarium from B. typhosus, as the former ferments promptly and uniformly.

Sorbite is promptly fermented by B. typhosus and by most of the paratyphoid group, which agrees with the findings of other observers. The intermediate avian types show their relationship in the tendency to suppression of this ability. B. pullorum, although fermenting uniformly, does so slowly. B. sanguinarium is not fixed in relation to this fermentation, and the results resemble to some extent those of B. typhosus on arabinose, dulcite, and xylose. Without due regard to the question of low or latent avidity, differential value could be attributed erroneously to such findings. The temporal differences in fermentation between B. typhosus and B. sanguinarium are, however, of differential value.

Maltose fermentation, as noted by Smith and Ten Broeck,[8] differentiates between B. sanguinarium and B. pullorum. Our results are the same. With prolonged incubation, however, B. pullorum shows irregular evidences of fermentation in from sixteen to forty days. As maltose is notably subject to hydrolysis, the prolonged incubation rather than a latent ability to ferment may explain these findings.

Rhamnose fermentation evidently differentiates B. typhosus from both the aerogenic and anerogenic paratyphoid types. B. sanguinarium, however, shows its close relationship to B. typhosus in the lower avidity exhibited for this sugar. The ability to produce gas from rhamnose was also observed with some strains. This was found to be irregular and unreliable and differences in gas production were without significance. Although some observers have induced B. typhosus to ferment rhamnose, from our own observations we feel that for all practical purposes the differentiation given is fundamentally valid. Muller [12] described the formation of fermenting papillæ showing acid change of the indicator on typhoid colonies when grown on rhamnose-agar and considered this a mutation specific for the organism Penfold [10] observed acid production by typhoid strains when grown in rhamnose peptone water for seven to twenty-one days, which results are not in accord with our observations.

Rhamnose has been frequently referred to as "iso-dulcite," but as it is a methylated pentose, this term, indicating an alcohol structure, is inappropriate and confusing.

With the apparently basic characteristic of acid production from rhamnose, we may tentatively define the limits of the paratyphoid-enteritidis group. To be included in this group an organism must produce acid from rhamnose, but not from salicin, saccharose or lactose; the ability to produce gas from glucose is a secondary characteristic.

In regard to B. pullorum and B. sanguinarium, which are of interest because of their close antigenic relationship to B. typhosus, the differences can be summarized as follows (Table II.). The time limits for differentiation are noted where these differences are temporal only, and the rapidity with which the difference is elicited, where the difference is qualitative:

TABLE II.

Type.	Gas from Glucose.	Acid Production from				
		Maltose (prompt).	Dulcite (48 hrs.).	Arabinose (prompt).	Rhamnose (slow).	Sorbite (48 hrs.).
B. typhosus	—	+	—	—	—	+
B. sanguinarium	—	+	+	+	+	—
B. pullorum	+	—	—	+	+	+ or —

Gas production by B. pullorum may be suppressed, as shown by Smith and Ten Broeck.[8] We are indebted to them for the strain which they found did not produce gas from glucose. In our hands this strain has resumed its ability to produce gas, showing the tendency to variation in this characteristic. The differences in maltose fermentation given in the table, as has been stated, were observed by them. Since the completion of our work Rettger and Koser [13] have reported a comparative study of B. sanguinarium and B. pullorum and found that dulcite, and also dextrine, separates these two types. B. typhosus was not included in their study.

We were led to repeat the agglutinative observations of Smith and Ten Broeck, since the agglutinative similarity of B. typhosus, B. sanguinarium, and B. pullorum, associated with marked cultural differences, parallels our findings [14] with other members of the paratyphoid group, some of which showed equally marked cultural differences with apparent agglutinative identity. The results are summarized in Table III. Only one dilution is recorded, the highest in which equally complete reactions were found for the three types.

TABLE III.

Type.	Typhoid Serum (Horse).	Fowl Typhoid Serum (Rabbit).	B. Pullorum Serum (Rabbit).
B. typhosus	6,000	1,250	1,000
B. sanguinarium	800	1,500	1,000
B. pullorum	800	1,500	1,250

In general, the results are similar to those of Smith and Ten Broeck as well as to those of Rettger and Koser. Our results with typhoid serum, however, differ from those of the former observers in the very distinctly lower cross-agglutination of B. sanguinarium and B. pullorum. Because of this difference two other horse sera were tried with the same results. The differences noted, however, with our sera were paralleled by the absorption results of Smith and Ten Broeck, who found that the avian strains did not remove the specific agglutinins for B. typhosus from typhoid sera. The high degree of cross-agglutination recorded by these workers is not, therefore, constant with all sera, but if present, absorption methods will still differentiate.

The other anaerogenic strains also showed no agglutinative differences, although as is evident in Table I., they differed somewhat among themselves in their fermentative reactions and sharply from the type strain of B. paratyphosus "B" included as a control. The agglutinative results (macroscopic method) are as follows:

TABLE IV.

Strains.	Sera and Dilutions.						
	B. paratyphosus A.		B. paratyphosus B.			B. enteritidis.	
	1-1,000	1-5,000	1-2,000	1-4,000	1-7,000	1-1,000	1-5,000
B.paratyphosusB.No.93,	—	—	+++	++	++	—	—
B. suis (Dorset)........	—	—	+++	++	++	—	—
B. suis No. 152.........	—	—	+++	++	++	—	—
Ten Broeck:							
Gas—...............	±	—	++++	+++	++	—	—
" +...............	±	—	+++	+++	++	—	—
Controls:							
A type..............	++++	++	—	—	—	—	—
B type..............	—	—	++++	+++	++	—	—
B. enteritidis.........	—	—	—	—	—	++++	+++

++++Complete agglutination.

SUMMARY.—The ability to produce acid from rhamnose is the essential characteristic of the paratyphoid-enteritidis group, differentiating both the aerogenic and anaerogenic members from B. typhosus. Additional cultural differences between B. typhosus, B. sanguinarium, and B. pullorum are presented. The agglutinative relationship of the strains studies is recorded. Observations are added on the low or latent avidity for carbohydrates in relation to variability and practical differentiation. Without due regard to these factors, erroneous differential significance might easily be given to variation even among members of the fixed groups.

REFERENCES.

NOTE.—An acknowledgment is due Dr. W. W. Ford, who called our attention to his paper, "The Carbohydrate Reactions of the Paratyphoid or Paracolon Group. (Preliminary Communication)," Medical News, June 17, 1905. He determined gas production from a series of carbohydrates by members of this group. Although some of his conclusions as to inability to ferment, being based on gas production only, are not valid, two striking results were obtained. He noted that his "A" and "B" strains differed on xylose. He was therefore the first to note this difference. Unaware of his findings, our results with a large series of cultures constitute an independent confirmation. He also found that his "hog-cholera" strains failed to produce gas from arabinose and suggested that this was of differential value in separating this group. Various workers have employed this carbohydrate and although differences have been noted no similar significance has been attributed by them to these findings. It is of interest to note that the differential value suggested by Ford has been found to hold for many, but not all of the "suis" strains we have studied when employing acid production as an indication of fermentation. His results with xylose have been overlooked, not only by us, but by all who have worked on this group of bacteria during the last ten years. Evidently, had his work been noted and repeated, the significance of differences in xylose fermentation would soon have been generally known.

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STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

V.* The Correlation of Culture and Agglutination Results, with Special Reference to B. Paratyphosus "B" and B. Cholerae Suis.

Charles Krumwiede, Jr., Lawrence A. Kohn, and Eugenia Valentine.

In a previous communication[1] we reported, in a tentative way, a series of observations on the reaction, in a glucose serum water medium, of a series of strains of the paratyphoid-enteritidis group together with their fermentative results. Although some of the differences elicited were paralleled by agglutinative differences to only a limited extent, they did correlate closely with host origin, that is, the animal species from which the strains were isolated. For the latter reason they seemed of sufficient interest to report, pending the preparation of sera for a more detailed agglutinative and agglutinin-absorption study.

The reaction of the strains in the glucose-serum-water medium mentioned was obtained in a medium prepared as follows: To 400 cubic centimeters of sterile distilled water add 100 cubic centimeters of sterile horse serum, five cubic centimeters of the Andrade indicator and sufficient sterile twenty per cent glucose solution to give a sugar content of 0.1 per cent. Fill in test-tubes from a sterile covered funnel and heat in the Arnold sterilizer on two days for ten to twenty minutes. Before use, a batch of medium should be tested with two known strains to determine its suitability.

In their growth the inoculated paratyphoid strains will differ as to whether they can or cannot decolorize the dye, the color of which has been returned by the acid produced from the trace of glucose present. When observed after eighteen hours the medium is usually coagulated and deep pink with non-decolorizing strains and pale pink to colorless with strains capable of decolorizing the dye. Intermediate gradations are noted occasionally with old stock strains. This difference in the final color of the inoculated medium we attributed to the ability or inability of strains of the paratyphoid group to reduce the fuchsin. Although this still seems to be the basic reason for the difference, a recent report makes us question whether secondary alkalinization may not contribute to this change to a greater extent than we thought. We have used the medium for three years with, on the whole, satisfactory and comparatively uniform results. Just recently, however, several samples of sera have given unsatisfactory color differences. The factors involved in the reaction, as well as the reasons for the difficulties with this medium, are given more in detail after presenting the general results.

The observations on fermentative differences as originally given have been extended, and although the final results have been practically the same, some revision in interpretation is necessary, due to later observations.

In a study[2] of the fermentative characteristics of the typhoid bacillus and the very closely allied paratyphoid types of fowl origin, we encountered results indicating that latent or semi-latent fermentative capacities were much more common and more important from the standpoint of practical differentiation, than they had seemed at first. We also encountered fermentative differences within groups of organisms, which according to all the other evidence available, are homogeneous. Such fermentative differences within a group are probably due to the development by some strains of a latent capacity possessed by all. This factor must be carefully considered before assigning classifying significance to a fermentative difference.

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* Published in Jour. Med. Res., 1918, 38, p. 89.

[1] Study III, Some Cultural Characteristics and Their Relation to Host Origin, Journ. Med. Res., 1917, xxxv, 357, is not reprinted. This report was a preliminary note on the cultural and fermentation reactions which are given fully and with further elaboration in this report.

The following table (I.) gives a complete summary of the cultural differences we have noted among the paratyphoid-enteritidis strains thus far studied. The results with litmus-milk are not included as they have been fully given.[3] The sharply defined differentiation of B. paratyphosus "A," due to its inability to ferment xylose as previously reported,[4] has been verified with all the strains which have been studied since that time. The varying degree of avidity for a carbohydrate exhibited by groups or among members of the same group is indicated. This brings out the factor of latency of some of the fermentative characteristics. The number of strains studied is also noted and the individual strain numbers are given where necessary for comparison with the agglutination results in the tables that follow. It will be noted that every strain studied culturally has not been included in the agglutination study.

TABLE I.

CULTURAL REACTIONS.

Strains.	Gas from Glucose.	Glucose-serum Water.	Acid Production, Peptone Water with 1% of:						
			Rhamnose.	Xylose.	Arabinose.	Dulcit.	Inosite.	Maltose.	Sorbite.
B. typhosus (37)	—	+	—	+ or ± [1]	— or ± [2]	± or — [3]	—	+	+
B. sanguinarium (5)	—	Irreg.	± [4]	+ to ± [5]	+	+ to ± [6]	—	+	± or — [7]
B. pullorum (13)	+ (—)	Irreg.	+	+ to ± [8]	+	—	—	— [9]	± [10]
B. paratyphosus A (2) B. paratyphosus A (78)	+	— [11] +	+	—	+	±	—	+ [12]	+ [12]
B. abortus equi (12)	+	+	+	+	+	+	—	+	+
B. choleræ suis (11)[13] B. choleræ suis (5)[14] B. choleræ suis (9)[15]	+ (—)	+	+	+	— [16]	— — or ± [17] ± or + [17]	—	+ (±)	+
Type ? [18] (3)	+ (—)	+ (±)[19]	+	+	—	± or +	—	+	+
B. choleræ suis (1)[20]	+	+	+	+	± or —	+	—	+	+
B. choleræ suis (2)[21]	+ (—)	—	+	+	+	+	+	+	+
B. paratyphosus "B" (36)[22] B. paratyphosus "B" (1)[23]	+	— ±	+	+	+	+ [24]	+	+	+
B. caviæ (8)[27] B. murium (5)	+	—	+	+	+	+	+	+	+
Type ? (2)[25] Type ? (2)[26]	+	—	+	+	+	+	— or ± —	+	+
B. caviæ (7) B. murium (3)	+	—	+	+	+	+	—	+	+
B. enteritidis (B. danysz) (14)	+	—	+	+	+	+	—	+	+

EXPLANATION AND NOTES TO TABLE I.

Symbols:	Gas production,	(—)	= absent with occasional stock strains.
	Serum water,	+	= deep pink color.
		+	= moderate color.
		∓	= pale or colorless.
	Acid production,	+	prompt change of indicator, evident within 24 hours.
		+	= later or delayed.
		∓	= no change after 21 days.
	Number,	( )	= number of strains studied.

NOTES:

Xylose and inosite,	Occasional slightly delayed results have been noted in groups given as +. As the general avidity of the group was high these have not been noted, to avoid further complexity of the table.
B. typhosus,	[1] xylose, 29 gave acid in 24 hours, 8 acid on the 5th and 13th day.
	[2] arabinose, 2 gave acid very late or were negative.
	[3] dulcit, gave acid on 5th to 21st day when positive.
B. sanguinarium,	[4] rhamnose, acid on 2d to 5th day.
	[5–6] xylose and dulcit, acid on 1st or 2d day.
	[7] sorbite, acid on 9th to 14th day, some strains irregularly positive, others gave only negative results.
B. pullorum	[8] xylose, acid, 1st or 2d day.
	[9] maltose, change of indicator noted after long incubation, due to inversion of sugar at incubator temperature?
	[10] sorbite, acid on 2d to 7th day.
B. paratyphosus "A,"	[11] stock strains, uniformly + with fresher strains.
	[12] only moderate number of strains studied on these carbohydrates; one B. suis strain, slowly positive on maltose.
B. choleræ suis,	[13] stock strains Nos. 111, 136, 152, 153, 157, 169, 170, 155; recently isolated, 303, 304, 308.
	[14] stock strains Nos. 125, 127, 131, 159, 120.
	[15] stock strains Nos. 118, 128, 124, 134, 235; recently isolated, Nos. 301, 302, 305, 307.
	[16] occasional very late change in indicator, fermentation?
	[17] same strain may give negative or positive results, where uniformly positive, varies widely in rate.
Types (?),	[18] stock strains Nos. 56, 93, 54 (in fractions a and b) presumably of human origin, received labeled as para, ' A."
	[19] two fractions of No. 54 give different results.
B. choleræ suis,	[20] recently isolated strain No. 300.
	[21] stock strains Nos. 112 and 113 labeled B. suis.
B. paratyphosus "B,"	[22] stock strains Nos. 52, 53, 59, 78, 95, 97, 100, 101, 149, 178, 900, 1100, 1500 and 2000; recently isolated, Nos. 222, 223, 224, 225, 226, 227, 229, 231, 232, 233, 234, 237, 238, 246, 274, 275, 284, 294, 295, 296, 297, and 298. More recent strains known to be from paratyphoid fever.
	[23] stock strain No. 105.
	[24] dulcit, acid may be very slightly delayed.
	[25] stock strains 94 and 96 presumably human origin.
	[26] stock strains 1200 and 1800 presumably of human origin.
	[27] the strains given as B. caviæ from guinea-pig infections, B. (typhi) murium from mice, and the B. enteritidis group have not been fully identified. They are given as such according to source.

The agglutinative results (Table II.) with some of the above groups have been so definite that a study of the absorption capacity of these strains was not undertaken, especially as these groups are generally accepted as agglutinatively distinct. The results with B. abortus equi accord with those of Meyer[5] and others, who have shown that this type is a distinct agglutinative entity. In the case of B. sanguinarium and B. pullorum, the unusual degree of cross-agglutination reveals the very close relationship of these types. The available evidence, however, indicates that they are separate groups. The agglutinative results given for these types are similar to those of Smith and Ten Broeck,[6] and Rettger and Koser.[7] This leaves for further study the groups of B. paratyphosus "B," B. (choleræ) suis, and similar types from rodents, and B. enteritidis and B. danysz. The agglutinative data that is presented below is limited to B. paratyphosus "B" and B. suis types. A sufficient number of sera are not available for an adequate study of the other types.

TABLE II.

AGGLUTINATION RESULTS.*

Immune Sera against—	Groups of Bacilli.				
	B. Typhosus.	B. Sanguinarium.	B. Pullorum.	B. Paratyphosus "A."	B. Abortus.
B. typhosus	+	±[3]	±[3]	—	—
B. sanguinarium	±	+	+	—	—
B. pullorum	±	+	+	—	—
B. paratyphosus "A,"	—	—	—	+ (—)[1]	—
B. abortus equi	—	—	—	—	+
B. paratyphosus "B,"	—	—	—	—	—
B. enteritidis[2]	—	—	—	—	—

*Cross-agglutination not noted when evidently within the average range of action of group or normal agglutinins.

[1] A few strains culturally "A" types are not agglutinated by "A" serum or by any of the sera for these groups.

[2] Not all the strains in this group have been identified agglutinatively. They are included in the history and cultural reactions of the strains.

[3] Cross-agglutination much more marked with some sera. These types do not absorb typhoid agglutinins from typhoid serum (Smith and Ten Broeck).

The following tables (III. and IV.) give the agglutination results with the strains of B. paratyphosus "B," B. suis, and the few strains which fall with these types in their cultural reactions. The agglutinations have all been carried out macroscopically, using eighteen-hour broth cultures to which 0.1 per cent of formalin was added. Dilution by an equal bulk of formalin-salt solution usually gave a satisfactory density. If the broth culture was heavy further dilution was done, till the density was sufficiently reduced. On the results of direct agglutination, the evidence is strongly indicative of the existence of two distinct groups. The degree of cross-reaction varies greatly with different sera, thus B. suis serum No. 169 agglutinates the "B" types slightly, whereas serum No. 56 shows very marked cross-agglutination. The four strains of human origin, Nos. 94, 96, 1,200 and 1,800 are presumably "B" types. They are rather sharply separated from the other strains, however, by their marked lack of sensitiveness to group agglutinins. Two stock culture strains (Nos. 112, 113), with a history of porcine origin, resemble the "B" types on direct agglutination and culturally. Most of the B. suis types fall together, and no agglutinative differences are noted, whether or not the strains ferment dulcit or arabinose. Three strains (Nos. 56, 93, 54), with a history of human origin which may be correct, in that they were isolated from cases of meat poisoning, apparently are B. suis types. The separation into two groups by direct agglutination correlates with the general tendency to separate into two cultural groups, according to the reaction in glucose-serum water, and the fermentation reactions. Several porcine strains fall into neither group, being influenced slightly or not at all by any of the agglutinative sera.

Strains.	Cultural Grouping.				Agglutinative Sera[2] according to Homologous Strain Number.																																
		Acid from—			"B" type No. 178.				"B" type No. 225.				B. suis No. 169.					B. suis No. 118.					B. suis No 131.					Type (?) No. 56.				
	Gluc. serum Water.	Arabinose.	Dulcit.	Inosite.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.
"B" type 59	—	+	+	+	+	+	+	±	+	+	+	±	+	+	+	+	±	+	+	+	+	±	+	+	+	+	±	+	+	+	+	+
78	"	"	"	"	+	+	+	+	+	+	+	±	+	+	±	±	×	+	+	+	±	×	+	+	±	±	×	+	+	+	+	±
97	"	"	"	"	+	+	+	+	+	+	+	±	+	±	×	—	—	+	+	±	×	—	+	+	+	+	±	+	+	+	+	+
100	"	"	"	"	+	+	+	+	+	+	+	+	—	—	—	—	—	+	±	—	—	—	×	×	—	—	—	+	+	+	±	±
178	"	"	"	"	+	+	+	±	+	+	±	×	±	×	—	—	—	+	±	×	—	—	±	×	×	—	—	±	±	±	×	×
222	"	"	"	"	+	+	+	±	+	+	+	+	×	—	—	—	—	+	+	±	—	—	±	±	±	×	×	+	+	+	+	+
223	"	"	"	"	+	+	+	±	+	+	+	+	×	—	—	—	—	+	+	×	—	—	±	±	×	×	—	+	+	+	+	±
224	"	"	"	"	+	+	+	+	+	+	+	+	×	—	—	—	—	+	+	±	×	—	±	±	×	×	—	+	+	+	+	±
225	"	"	"	"	+	+	+	+	+	+	+	+	×	×	—	—	—	+	±	×	—	—	±	±	×	×	×	+	+	+	+	+
226	"	"	"	"	+	+	+	+	+	+	+	+	×	—	—	—	—	+	±	×	—	—	+	±	±	×	×	+	+	+	+	±
227	"	"	"	"	+	+	+	+	+	+	+	+	×	—	—	—	—	+	±	×	—	—	×	×	—	—	—	+	+	+	+	±
229	"	"	"	"	+	+	+	+	+	+	+	±	+	+	±	×	×	+	+	+	±	×	±	±	±	×	×	+	+	+	+	+
231	"	"	"	"	+	+	+	+	+	+	+	±	+	+	±	×	×	+	+	+	±	×	+	+	±	±	×	+	+	+	+	+
232	"	"	"	"	+	+	+	+	+	+	+	+	×	×	—	—	—	+	+	±	×	—	+	+	±	×	×	+	+	+	+	+
233	"	"	"	"	+	+	+	±	+	+	+	+	×	×	—	—	—	+	±	×	—	—	±	±	×	×	—	+	+	+	+	±
234	"	"	"	"	+	+	+	+	+	+	+	+	×	×	—	—	—	+	±	×	—	—	±	±	×	×	—	+	+	+	+	+
246	"	"	"	"	+	+	+	+	+	+	+	+	×	—	—	—	—	+	+	×	—	—	±	±	×	×	—	+	+	+	+	+
274	"	"	"	"	+	+	+	+	+	+	+	+	±	×	×	—	—	+	+	±	×	×	±	±	±	×	—	+	+	+	±	×
275	"	"	"	"	+	+	+	+	+	+	+	+	×	—	—	—	—	+	±	×	—	—	±	±	±	×	×	+	+	+	+	±
105	±	"	"	"	+	+	+	+	+	+	+	+	±	×	×	—	—	+	+	±	×	—	+	+	±	±	×	+	+	+	+	+
B. suis 112	—	+	+	+	+	+	+	±	+	+	+	+	±	×	—	—	—	±	±	×	—	—	±	±	×	×	×	+	+	+	+	±
113	"	"	"	"	+	+	+	±	+	+	+	+	±	±	×	—	—	±	±	×	×	—	+	+	±	×	×	+	+	+	+	+
Type (?) 94	—	+	+	— or ±	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
96	"	"	"	"	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	±	±	×	—
1,200	"	"	"	—	+	+	+	±	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,800	"	"	"	"	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—

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[1] None of these strains agglutinated in B, paratyphosus "A" or in B, enteritidis sera beyond the range of group or normal agglutinins.

[2] Sera against Strains Nos. 178, 225, 131, and 56 were obtained from young goats; sera for Strains Nos. 169 and 118 were from rabbits. The bacilli were injected intravenously, starting with killed organisms, followed later by live.

Symbols: + = complete agglutination; ± = partial agglutination; × = slight agglutination

TABLE IV.

AGGLUTINATION RESULTS.[1]—Continued.

Strains.	Cultural Grouping.				Agglutinative Sera according to Homologous Strain Number.																																	
	Gluc.-serum Water.	Acid from—			"B" type No. 178.				"B" type No. 225.				B. suis No. 169.					B. suis No. 118.					B. suis No. 131.					Type (?) No. 56.				
		Arabinose.	Dulcit.	Inosite.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.
B. suis 111....	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	×	—	—	—	+	+	±	×	—
136....	“	“	“	“	+	+	±	×	+	±	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
152...	“	“	“	“	+	+	±	×	±	±	×	—	+	+	+	+	±	+	+	+	+	±	+	+	+	+	±	+	+	+	+	+
153....	“	“	“	“	+	+	±	×	+	±	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
155....	“	“	“	“	+	+	±	×	±	±	×	×	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±
169....	“	“	“	“	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	±	×	+	+	+	±	×
170....	“	“	“	“	+	+	×	—	+	±	—	—	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
303....	“	“	“	“	+	+	×	—	±	×	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
304....	“	“	“	“	+	+	+	±	+	±	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
308....	“	“	“	“	+	+	+	±	+	±	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

120....	“	“	— or ±	“	+	+	±	+	±	×	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
131....	“	“	“	“	+	+	±	×	±	×	—	—	+	+	+	+	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
159....	“	“	“	“	+	+	±	×	+	+	±	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
118....	“	“	± or +	“	+	+	±	×	±	±	×	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
124....	“	“	“	“	+	±	±	×	+	±	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
128....	“	“	“	“	—	—	—	—	—	—	—	—	+	±	±	×	—	±	×	—	—	—	+	±	±	×	—	±	±	×	—	—
134....	“	“	“	“	+	+	×	×	×	—	—	—	+	+	+	+	+	+	+	+	+	±	+	+	+	±	×	+	+	+	±	±
235....	“	“	“	“	±	±	×	×	×	×	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
301....	“	“	“	“	+	+	±	×	+	±	×	—	+	+	+	+	±	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
302....	“	“	“	“	±	±	×	—	±	×	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
305....	“	“	“	“	×	×	—	—	×	×	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
307....	“	“	“	“	+	+	±	×	+	±	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Type (?) 56....	+	—	± or +	—	+	+	×	×	±	×	—	—	+	+	+	+	±	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+
93....	“	“	“	“	+	+	±	×	+	±	×	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54b...	“	“	“	“	+	+	±	×	±	±	—	—	+	+	+	+	±	+	+	+	+	±	+	+	+	±	×	+	+	+	±	×
54c...	±	“	“	“	+	+	±	×	+	±	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. suis 300....	+	± or —	+	—	+	+	+	±	+	±	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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[1] None of these strains were agglutinated by B, paratyphosus "A" or B, enteritidis sera beyond the range of group of normal agglutinins.

A study of the tables (V. and VI.) giving the results of absorbing the agglutinins from the various sera shows a marked parallelism with the direct agglutinative results. In some instances the absorption of a serum by a strain of the opposite group has not removed all the group agglutinins for some of the strains in the same group. The differences between the two groups, even in these instances, were so sharp that further absorption seemed unnecessary.

TABLE V.

AGGLUTININ-ABSORPTION RESULTS.

Strains	Cultural Grouping.				"B" serum No. 178 absorbed by "B" type No. 178.				"B" serum No. 225 absorbed by "B" type No. 225.				"B" serum No. 178 absorbed by B. suis No. 169.				"B" serum No. 225 absorbed by B. suis No. 131.				Suis serum No. 131 absorbed by B. suis No. 131.				Suis serum No. 169 absorbed by "B" type No. 178.						Suis serum No. 118 absorbed by "B" type No. 178.						"B" serum No. 225 absorbed by type (?) No. 56.				Suis serum No. 131 absorbed by type (?) No. 56.			
	Gluc.-serum Water	Acid from—																																										
		Arabinose.	Dulcit.	Inosite.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-1,000.	1-2,000.	1-4,000.	1-150.	1-1,000.	1-2,000.	1-4,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-150.	1-500.	1-1,000.	1,2000.	1-4,000.	1-7,000.	1-150.	1-1,000.	1-2,000.	1-4,000.	1-150.	1-500.	1-1,000.	1-2,000.
"B" type 59	—	+	+	+									+	+	+	±	+	+	±	—	—	—	—	—	+	+	±	±	×	—	+	+	±	×		—	+	+	±		±			—
78	"	"	"	"								—	+	+	+	±	+	+	+	±	—				+	±	×				+	±	×			—	+	+	±	×	±			
97	"	"	"	"	×		—		—	—		—	+	+	+	±	+	+	±	×	—	—	—	—	—	—		—	—	—	—		—	—		—	+	+	+	+	×			—
100	"	"	"	"	—	—	—	—	—	—	—	—	+	+	+	±	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—
178	"	"	"	"	—	—	—	—	—	—	—	—	+	+	+	±	+	+	±		—				—	—			—		—			—		—	+	+	±		±	—	—	—
222	"	"	"	"	—	—	—	—	—			—	+	+	+	±	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		+	+	+	+	—	—	—	—
223	"	"	"	"	—		—	—		—			+	+	+	±	+	+	+	+			—			—					—		—	—	—	—	+	+	+	+			—	
224	"	"	"	"					—				+	+	+	+	+	+	+	+		—	—	—	—	—	—		—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—
225	"	"	"	"	—	—	—	—	—	—			+	+	+	+	+	+	+	+		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—
226	"	"	"	"	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	±				—		—					—		—	—	—	—	+	+	+	+	—	—	—	—
227	"	"	"	"	—	—	—						+	+	+	±	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		+	+	+	+	—		—	—
229	"	"	"	"					—		—	—	+	+	+	+	+	+	±	×	—	—	—		+	+	±	×			+	+	×	×	—	—	+	+	+	+	±			
231	"	"	"	"	—	—	—	—	—	—		—	+	+	+	+	+	+	+	±	—			—	+	+	±	×	×		+	+	±	×			+	+	±	×	—		—	—
232	"	"	"	"	—	—	—	—	—	—		—	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—		—
233	"	"	"	"	×	—	—	—	—	—		—	+	+	+	±	+	+	+	+				—	—	—	—	—	—	—	—	—		—	—	—	+	+	+	+	—	—		
234	"	"	"	"	—	—	—	—	—	—			+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	+	+	+	+	—	—		—
246	"	"	"	"	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	—	—			—	—		—	—	—	—	—	—	—	—	—	+	+	+	+	—	—		
274	"	"	"	"	×	—	—	—					+	+	+	+	+	+	+	±	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	+	+	+	+	—		—	—
275	"	"	"	"	—	—	—		—	—	—	—	+	+	+	+	+	+	+	+	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	+	+	+	+	—		—	
105	"	"	"	"	×	—	—	—	—	—	—	—	+	+	+	+	+	+	±	×	—	—			—	—		—	—	—	—	—	—	—	—	—	+	+	+	±	—	—	—	—
B. suis 112	—	+	+	+	—	—	—		—	—	—	—	+	+	+	±	+	+	+	±	—	—	—	—	+	±	±	×	—	—	+	±	±	±	×	—	+	+	+	+	±	—	—	—
113	"	"	"	"	—		—		—	—	—	—	+	+	+	±	+	+	+	+	—		—	—	+	±	±	±	×		+	+	±	±	×	×	+	+	+	+	±		—	—
Type (?) 94	—	+	+	or ±	—	—	—	—	—	—		—	+	+	+	+	+	+	+	+		—				—	—		—	—	—	—		—	—	—	+	+	+	+	—	—	—	
96	"	"	"	"	—	—	—	—	—		—	—	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		+	+	+	+	—	—	—	—
1,200	"	"	"		—	—	—	—	—	—	—	—	+	+	+	±	+	+	+	+	—		—	—	—			—	—	—			—	—	—	—	+	+	+	+	—	—	—	—
1,800	"	"	"	"	—	—	—	—	—		—	—	+	+	+	±	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—		—	—

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TABLE VI.

AGGLUTININ-ABSORPTION RESULTS.—Continued.

Strains.	Cultural Grouping. Gluc.-serum Water.	Cultural Grouping. Acid from— Arabinose.	Dulcit.	Inosite.	"B" serum No. 178 absorbed by "B" type No. 178. 1-150.	1-500.	1-1,000.	1-2,000.	"B" serum No. 225 absorbed by "B" type No. 225. 1-150.	1-500.	1-1,000.	1-2,000.	"B" serum No. 178 absorbed by B. suis No. 169. 1-150.	1-500.	1-1,000.	1-2,000.	"B" serum No. 225 absorbed by B. suis No. 131. 1-150.	1-500.	1-1,000.	1-2,000.	Suis serum No. 131 absorbed by B. suis No. 131. 1-150.	1-500.	1-1,000.	1-2,000.	Suis serum No. 169 absorbed by "B" type No. 178. 1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	Suis serum No. 118 absorbed by "B" type No. 178. 1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	"B" serum No. 225 absorbed by type (?) No. 56. 1-150.	1-500.	1-1,000.	1-2,000.	Suis serum No. 131 absorbed by type (?) No. 56. 1-150.	1-500.	1-1,000.	1-2,000.
B. suis 111.	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
136	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	±	—	—	—	—	—	—	—	—
152	"	"	"	"	—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	±	—	—	—	—	—	—	—	—
153	"	"	"	"	—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	×	—	—	—
155	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	±	—	—	—	—	—	—	—	—
169	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	+	—	—	—	—	×	—	—	—
170	"	"	"	"	—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	×	+	+	+	+	+	—	—	—	—	—	—	—	—
303	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	+	—	—	—	—	—	—	—	—
301	"	"	"	"	—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
308	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
120.	"	"	— or ±	"	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	+	—	—	—	—	×	—	—	—
131	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	×	+	+	+	+	+	—	—	—	—	—	—	—	—
159.	"	"	"	"	—	—	—	—	—	—	—	—	+	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
118	"	"	± or +	"	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	±	—	—	—	—	—	—	—	—
121	"	"	"	"	—	—	—	—	—	—	—	—	+	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
128	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	±	×	—		×	—		—	—	—	—	—	—	—	—	—	—
131	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	±	—	—	—	—	—	—	—	—
235	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
301	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	+	—	—	—	—	—	—	—	—
302	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
305	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	+	—	—	—	—	—	—	—	—
307	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Type (?) 56	+		± or +	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	±	×	—	—	—	—	—	—	—	—
93	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
51b	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	×	+	+	+	±	±	—	—	—	—	—	—	—	—
51c.	"	"	"	"	—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
B. suis 300.	+	± or —	+		—	—	—	—	—	—	—	—	±	±	×	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—

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Because Strain No. 56 stimulated the production of group agglutinins to such a marked extent, it was used to absorb both the " B " type and B. suis sera. Evidently this strain, with a history of human origin, is a typical B. suis type in its absorptive capacity. As mentioned above, the source given may be correct, in that it was isolated from a case of food poisoning.

Two strains of porcine origin are noteworthy, Nos. 112 and 113. Culturally and on direct agglutination they resemble closely the " B " types, and the latter types also absorb the agglutinins of " B " sera active against these two strains. When B. suis sera were absorbed with a " B " type, however, there was little if any reduction in the group agglutinins active against these two strains. On repetition and with direct comparison with the same dilutions of the unabsorbed sera, no appreciable difference could be noted between the absorbed and the unabsorbed sera.

These results strongly indicate the presence of group agglutinins active against these strains which are different in kind from those active against the " B " types, and not absorbable by the latter. Further absorption experiments were, therefore, carried out. The results are given in Table VII. Some of the results already given are repeated in this table for comparison. These results show that according to their absorptive capacity the two strains belong neither to the " B " group nor to the B. suis group. The apparent discrepancies in absorption are confined to the absorption of group agglutinins, and are probably explicable on the basis that different types of group agglutinins are present in the various sera. The sharp fall of B. suis agglutinins in B. suis serum might be considered due to a rudimentary ability to bind B. suis agglutinins, that is, the strains may be true " suis " strains which have lost their ability of responding to B. suis agglutinins, but have not compeltely lost their ability to bind them. This is pure hypothesis, however. The dose of bacilli added was far in excess of a volume of a typical B. suis strain, which would suffice to completely absorb every trace of agglutinins.

TABLE VII.

AGGLUTININ ABSORPTION. B. SUIS Nos. 112 AND 113.

Strains.	"B" Serum absorbed by—																B. suis Serum absorbed by—																				
	"B" type.			B. suis.			B. suis No. 112.					B. suis No. 113.					"B" type.						B. suis.			B. suis No. 112.						B. suis No. 113.					
	1-150.	1-1,000.	1-2,000.	1-150.	1-2,000.	1-4,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-150.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-150.	1,500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.
"B" type 225.	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—	×	—	—	—	—	—
232.	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
B. suis 112....	—	—	—	+	+	±	—	—	—	—	—	—	—	—	—	—	+	±	±	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
113....	—	—	—	+	+	±	—	—	—	—	—	—	—	—	—	—	+	±	±	±	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Type (?) 94...	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
96...	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,200...	—	—	—	+	+	±	+	+	+	+	+	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,800...	—	—	—	+	+	±	+	+	+	+	±	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B suis 169...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	±	—	—	—	+	+	±	—	—	—
120...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	+	+	±	—	+	+	+	+	±	±
131...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	+	±	—	—	+	+	+	—	—	—
118...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	+	±	—	—	+	+	+	±	×	—
Type (?) 56..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	+	+	+	+	×	—
B suis 300...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	±	—	—	—	+	+	+	×	—	—

The reduction, therefore, is more probably referable to the excessive mass of bacilli used for the absorption, and the two strains cannot be classed with the typical B. suis strains. In one instance we added an excessive mass of B. paratyphosus "B" to a B. suis serum, and although the result was not a complete disappearance of B. suis agglutinins, the titer was reduced 90 per cent. This effect of an excessive mass of heterologous bacteria on the agglutinin content occurs not infrequently, according to the observations of others.[9] This phenomenon is analogous, apparently, to absorption by physical agents, such as kaolin or aluminium hydrate. To control this factor a record was kept for each absorption of the proportionate volume of bacilli to volume of diluted serum when finally sedimented in the centrifuge. When the proportion of packed bacilli to supernatant serum dilution was 1 to 4 or 3, or at most 1 to 2, satisfactory differential results were uniformly obtained. Only when the volume of bacilli equalled or exceeded the volume of supernatant serum dilution was a reduction encountered. Naturally these proportions would not hold for sera differing in titer from those we employed. All the absorptions were carried out by adding the serum diluted 1-15 to the packed bacilli, sedimented by centrifugalizing heavy suspensions, obtained by washing off the growth on agar in Blake bottles. By having a packed volume of bacilli, inadequate or excessive proportion of bacilli to diluted serum was avoided after the satisfactory limits had once been ascertained.

A study of the absorption results given, especially in relation to the lack of correlation with some of the cultural differences, raises the question whether absorption of the sera by strains showing such cultural differences would reveal any agglutinin-absorption differences. Table VIII. shows clearly that differences in fermentation of arabinose, or dulcit, among B. suis strains, and differences in fermentation of inosite, among B. paratyphosus "B" strains, have no classifying significance. Like differences in the fermentation of dulcit, among strains of B. typhosus, or differences in the fermentation of sorbite among strains of B. sanguinarium, the ability to ferment is probably, in some instances, an active manifestation of an otherwise semi-latent ability, or the inability to ferment may be the recession of a positive characteristic to a latent state.

TABLE VIII.

AGGLUTININ ABSORPTION OF STRAINS SHOWING CULTURAL DIFFERENCES.

Strains.	Cultural Grouping				B. Serum No. 225.												Suis Serum No. 131.															
	Gluc.-serum Water.	Acid from—			Absorbed by "B" type No. 225.				Absorbed by Type (?) No. 94.				Absorbed by Type (?) No. 1,200.				Absorbed by B. suis No. 152.				Absorbed by B. suis No. 131.				Absorbed by B. suis No. 124.				Absorbed by B. suis No. 300.			
		Arabinose.	Dulcit.	Inosite.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,200.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-150.	1-500.	1-1,000.	1-2,000.
"B" type 225	—	+	+	+	—	—	—	—	—	—	—	—	×	—	—	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
232	"	"	"	"	—	—	—	—	—	—	—	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Type (?) 94	"	"	"	± or —	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—	—	×	—	—	—	×	—	—	—
96	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,200	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,800	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B. suis 152	+	—	—	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
169	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—	—	—	×	—	—	—	—	—	—	—
131	"	"	— or ±	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B. suis 118	+	—	± or +	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
124	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Type (?) 56	"	"	"	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B. suis 300	"	± or —	+	"	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

In the B. suis group there are two strains which are influenced little, if at all, by B. suis sera. The question arises, are these B. suis strains which have lost the physical characteristic of reacting to B. suis agglutinins, or do they belong to separate groups? If the former were the case they might still be able to bind or absorb B. suis agglutinins. Table IX. shows that absorption of B. suis serum by Strain No. 111 has no effect on the agglutinin content of B. suis serum, but with No. 128 there is some reduction. The dose of absorbing bacilli, however, was very large. None of these strains, therefore, belongs to the group of typical B. suis strains.

TABLE 9.

ABSORPTION BY INAGGLUTINABLE B. SUIS STRAINS.

Strains.	Suis Serum 131 unabsorbed.					Suis Serum 131 absorbed by—											
						B. suis 111.						B. suis 128.					
	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-10,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.	1-150.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-7,000.
"B" type 225.	±	±	×	×	×	±	±	±	×	×	×	±	±	×	×	—	—
B. suis 131....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—

In Table X. the results obtained are summarized and given in a condensed fashion for ease of correlation and to bring out the dominent classificatory characteristics. The strains of human origin given in the preceding tables without type designation are included in the groups where they evidently belong, as shown by the agglutinin absorption results.

TABLE X.

SUMMARY OF STRAINS COMPLETELY STUDIED.

Groups.	No. of Strains.		Cultural Results.*				Agglutination.		Agglutination after Absorption.				Notes.
				Acid from:					"B" Serum absorbed by		Suis Serum absorbed by		
	Total.	Sub-group.	Gluc.-serum Water.	Arabinose.	Dulcit.	Inosite.	"B" Serum.	Suis Serum.	"B" type.	B. suis.	"B" type.	B. suis.	
B. paratyphosus "B".	24	1	±	+	+	+							
Human origin.....		19	—	"	"	"	+[2]	±[3]	—	+	—	—	Inosite negative strain absorbs "B" agglutinins.
		2	"	"	"	± or —							
		2	"	"	"	—							
B. suis............ Porcine origin...	2		—	+	+	+	+	±	—	+	±	—	Do not absorb "B" agglutinins.
B. suis............	26												
Human origin...		3	+[1]	—	± or +	—							

Porcine origin.......		9	“	“	—	“	±	+	—	—	+	—	Representative strains showing cultural differences absorb B. suis agglutinins.
“ “		3	“	“	— or ±	“							
“ “		8	“	“	± or +	“							
“ “		1	“	— or ±	+	“							
“ “		1	+[1]	—	—	—	—	—	—	—	—	—	
“ “		1	“	“	± or +	“	—	×[4]	—	—	×	—	Do not absorb B. suis agglutinins.

[1] One fraction of one strain reduces partially.

[2] + = to or near to the titer of the sera.

[3] ± = distinctly below the titer.

[4] × = influenced slightly.

* Symbols: Glucose-serum water, — = pale pink or colorless.
± = moderate pink.
+ = deep pink.
Acid production, + = prompt fermentation.
± = delayed fermentation.
— = no fermentation.

Condensing these results still further, Table XI. gives clearly the relative value of the several cultural reactions in relation to the separation of the groups. We have included in this final summary the strains not included in the agglutinin-absorption tables. The direct agglutinative results were sufficiently sharp to warrant their inclusion, especially in the light of the absorption results.

TABLE XI.

COMPARATIVE VALUE OF CULTURAL REACTIONS FOR DIFFERENTIATION.

Group.	Number of Strains.	Glucose-serum Water.	Acid Production from—		
			Arabinose.	Dulcit.	Inosite.
B. para. "B"........	41	40 —, 1 ±	41+	41+	37+, 2 ± or —, 2—
B. suis.............	27	27+ (1 fraction ±)	26 —, 1 ± or —	10 —, 17 — to +	27—
B. suis (not agglut.)*.......	2	2+	2—	1 —, 1 ±	2—
B. suis (intermediate types)......	2	2—	2+	2+	2+

*By B suis sera.

Evidently no one characteristic has served to differentiate between all of the B. suis and B. paratyphosus "B" strains. Dulcit fermentation, as with most other members of the group, varies according to sub-group avidity, and has differential value only in so far as a low avidity indicates that a strain does not belong to the "B" group. Inosite fermentation is evidently not a completely fixed characteristic, but has a high value where a positive result is obtained. The negative or delayed results were obtained with stock cultures, and it may be that with freshly isolated "B" strains all would give positive results, at least all the more recent strains we have studied have fermented this substance. With arabinose the tendency to group results is somewhat more sharp, but again the significance lies mainly in the value of slowly positive or negative results, indicating that a strain is not a "B" type. The glucose-serum water has given results coming nearest to an actual differential reaction available for separation of individual strains. Only two intermediate results are noted with old stock cultures, and in one of these instances two fishings from the same stock culture gave different results, showing the instability of this strain in relation to this reaction. Every recently isolated strain has, however, given results allowing immediate separation. Among B. paratyphosus "A" strains, likewise, we have encountered stock strains having the ability to reduce, but no recently isolated strain has had this ability, and the reaction has been uniformly successful in the differentiation from "B" types.

There is a general tendency to correlation between the different cultural characteristics, and although this correlation does not hold for individual strains, the tendency to group parallelism is added evidence of the validity of the grouping given. The two intermediate strains (B. suis Nos.

112 and 113) show this correlation strongly. If we look upon these strains as cultural variants from originally typical B. suis types, the acquisition of the ability to reduce the color in serum water has been associated with an increased avidity for the fermentable substances. If these strains are not cultural variants, they then form a sub-group, as do the other porcine strains uninfluenced by B. suis serum.

The study of two strains not included in the above tables indicates the value of glucose-serum water for presumptive differentiation. One strain was isolated by Bernstein and Fish [8] from a sample of pie, the cause of an outbreak of food-poisoning. This strain gave decolorization in the serum-water medium, and also fermented arabinose and dulcite, but not inosite, three of the four characteristics correlating. The first would have sufficed, however, to indicate that the strain was not a B. suis type. This strain, as shown by Bernstein and Fish, was not agglutinated by B. suis serum. Although this strain would not absorb all the agglutinins from " B " serum, a fact which we have verified, both this strain and B. paratyphoid "B " would remove the agglutinins from an autogenous serum. While not a typical " B " type, the strain is closely related, but has no relationship to B. suis, a fact indicated at once by the glucose-serum-water reaction. Another strain received as B. suis acted the same, and in the tabulation of the results was evidently a marked exception among our suis types. The strain, however, was found to agglutinate only in B. enteriditis serum, which correlated with and explained the apparently aberrant result.

To summarize: Two main groups, B. paratyphosus " B " and B. suis, have been differentiated on the basis of agglutination and agglutinin absorption. There is a strong correlation between the agglutination and cultural results. B. paratyphosus " B " gives decolorization with glucose-serum water, has a high degree of avidity for arabinose and dulcit, and usually ferments inosite. B. suis does not give decolorization with glucose-serum water, has a low avidity for or fails to ferment arabinose and dulcit, and does not ferment inosite. A few strains fall into neither group according to their agglutinative reactions, although culturally they belong to one or the other of the above groups. These last strains were received as of porcine origin.

The results obtained are offered as further evidence of the contention of Bainbridge,[9] that B. paratyphosus " B " and B. suis are distinct organisms. This has been disputed by Uhlenhuth [10] and others.

If B. paratyphosus " B " is a distinct organism, and is only isolated from man, then the epidemiology of paratyphoid fever due to this type is the same as for typhoid, that is, the disease is transmitted from man to man, either directly or indirectly through the excretions of those suffering from paratyphoid fever, or of carriers. If, however, it is not distinct from B. suis, then swine infected with this type must be considered as a possible source of infection, as well as individuals suffering from " meat poisoning," due to the ingestion of infected meat or other foods contaminated from this source, which contain bacilli as well as toxin; unless it could be shown that the two types differ in their pathogenicity.

Several factors probably enter into the lack of unanimity of opinion as to the existence of two groups. The study of stock cultures is likely to lead to false conclusions. The source and type of disease from which they were isolated may not be available, or the cultures may be wrongly labeled.

Even if a strain is known to have been the cause of an outbreak of food poisoning, or acute gastro-enteritis, it does not necessarily mean that it is a B. suis type. B. paratyphosus "B," like most members of the paratyphoid group, has a strongly developed ability to produce toxin, and the contamination of food from human sources cannot be excluded as an occasional cause of outbreaks of food poisoning. Bainbridge and Dudfield [11] have reported an outbreak of gastro-enteritis, due to B. paratyphosus "B." The relative lack of fixed fermentative characteristics has added to the difficulty, and has been used as evidence of variability. This group is notably subject to variation, and a stock culture may differ in many respects from its original characteristics. This may be discounted, but many have simply deduced from this variability the lack of validity of either cultural or agglutinative differences.

We shall not attempt a summary of the conflicting results obtained by English and German investigators. The views of the former are summarized by Bainbridge.[9] The opposite view is fully given by Uhlenhuth and Hübener,[10] in their summary on the paratyphoid group. A report by Jordan [12] appeared shortly after our previous paper, and while the present work was in progress. His results are directly comparable to our own in that they correlate agglutinative and cultural differences. He did not employ glucose-serum water or inosite, but the results obtained with xylose, arabinose, and dulcit, and the correlation of these results with agglutinative and absorption differences parallel our own very closely.

Two media suggested for the differentiation of B. paratyphosus "A" and "B" have been of immediate interest in relation to the differences elicited with glucose-serum water. One medium is lead acetate agar, which Burnet and Weisenbach[13] have employed with success. B. paratyphosus "B" causes browning of the agar, whereas B. paratyphosus "A" does not. The other medium is that suggested by Cole and Onslow.[14] The basis is a casein digest, with 0.2 per cent. glucose and phenol-sulphonephthalein as an indicator. In this medium B. paratyphosus "B" produces alkali in excess of the acid produced from the trace of glucose, as evidenced by the red (alkaline) color of the indicator. B. paratyphosus "A" gives an acid reaction, and the indicator is yellow. The proportion of volume of medium to diameter of tube must be considered or a maximum difference will not be obtained. A free supply of oxygen is necessary, or alkali production is interfered with.

The differences recorded by the latter workers are beyond question wholly dependent upon the end reaction of the medium. This raises the question whether we were in error in attributing the differences elicited with glucose-serum water to reduction. To determine this, we tried out other dyes as indicators in glucose-serum water, searching especially for those whose color change due to alkalinization could not be confused with the loss of color due to reduction. Two dyes, rosolic acid and indigo-carmine, have given us suggestive results. For comparison, phenol-sulphonephthalein was used as well. Different depths of medium have also been tried, and 0.2 per cent. glucose as well, so as to parallel the observations of Cole and Onslow. The following table gives the results. The findings with lead acetate agar are also included for comparison. Unless otherwise noted, columns of media from 1.5 to 3.5 centimeters gave similar results:

TABLE 12.

Groups.	Lead Acetate Agar.	Serum, 1 part; Distilled Water, 4 parts.				
		Glucose, 0.1%.	Glucose, 0.2%.			
		Andrade Indicator.[5]		Rosolic Acid, 0.01%.	Indigo Carmine, 0.01%.	Phenol-sulphone-phthalein.[1]
B. typhosus	+[4]	+	+	Salmon.	Blue.	Yellow.
B. sanguinarium	+ to —	Irreg.	Irreg.	Salmon.[2]	Blue.[2]	Yellow.
B. pullorum	+ to —	Irreg.	Irreg.	Irreg.	Irreg.	Yellow.
B. paratyphosus "A"[3]	—	+	+	Salmon.	Blue.	Yellow.
No. 86[3]	+	—	±	White.	Very pale blue.	"
No. 92	"	"	—	"		"
B. abortus	—	+	+	Salmon.	Blue to white.	Yellow.
B. suis	—	+	+	Salmon.	Blue.	Yellow.
No. 54b	"	"	"	"	White.	"
No. 54c	+	±	±	White.	"	"
B. paratyphosus "B"	+	—	—	White.	White.	Yellow.
No. 105	—	+	+	Salmon.	Blue.	"
B. caviæ	+	—	—	White.	White.	Yellow.
B. murium	"	"	"	"	"	"
B. enteritidis	+	—	—	White.	White.	Yellow.
B. danysz	"	"	"	"	"	"

NOTES: Rosolic acid medium, red before inoculation; on addition of 0.2% N/I HCl turns lavender pink, of 0.4% turns clear salmon.
Indigo carmine medium, clear blue before inoculation; moderate alkalinization does not change the color.
Phenol-sulphonephthalein medium, magenta red before inoculation, that is, the medium is alkaline to this indicator; on addition of 0.2% N/1 HCl turns yellow.

[1] 4 c.c. of a 0.04% solution per 100 c.c.

[2] One low column tube shows decolorization.

[3] Usual reaction for group, numbers refer to variant strains.

[4] Results after 24 hours' incubation; + = browning; — = no change.

[5] + = deep pink; ± = moderate pink; — = pale or colorless.

It is evident from the table that there is a marked tendency to parallelism of reaction in the various media, an exception being the serum water containing phenol-sulphonephthalein as an indicator. With this indicator the end reaction is uniformly acid. The loss of color with indigo-carmine is evidently due to reduction, as the color quickly returns when the decolorized tubes are shaken. The tendency to intermediate reactions, especially marked with the B. abortus group, is of interest in relation to results obtained with methylene blue. This dye, when employed with a few type strains, was uniformly reduced. The results with these two dyes indicate that a certain degree of resistance to decolorization is required to produce a sharp difference. The results with rosolic acid, which would remain red if alkalinization only occurred, are evidently due to reduction. The color, however, cannot be returned by shaking. The uniform acid reaction with phenol-sulphonephthalein and the parallelism of results with the other dyes, the loss of color with one being, without question, due to reduction, seems to us to indicate that the differences in serum water are due to the ability or the inability of the strains to reduce the indicator.

The results with rosolic acid were especially definite, giving fewer intermediate shades of color than did the Andrade indicator. Whether this would be a better indicator cannot be stated at present, as many batches of media would have to be tested to determine whether this superiority would be constant.

Some of the results with lead-acetate agar are the same as those just reported by Jordan and Victorson[15] who found that B. choleræ suis, like the "A" types, did not produce browning of the agar, whereas B. enteritidis reacted like the "B" types. It is of interest to note that the two "A" strains (see Table XII.), which vary in that they give reduction in serum water, also give browning with lead acetate, whereas the "B" type, which does not reduce, also fails to produce browning. With one B. suis strain the two fractions gave different results. Apparently the lead acetate reaction is susceptible to variation, and may lose its differential value when strains have been in cultivation for some time.

The reduction capacity of members of the paratyphoid group has been studied by previous observers.[10] In general, they found that B. paratyphosus "A" showed a lesser ability to reduce than the other members of the group. The work of Buchholz[16] is of special interest, as he found that, by using 0.3 to 0.5 per cent. agar, he could obtain sharper differences in reduction, thus differentiating B. paratyphosus "A" from the other more vigorously reducing members of the paratyphoid-enteritidis group. He obtained this difference with malachite green, with orcein, and with litmus as indicators. In a few instances it has been reported that B. choleræ suis failed to reduce, acting in this way like the "A" types. Others[10] denied this and claimed that there were no reduction differences between strains of human and animal origin. As these observations were limited in number and were not correlated with other group differences and as some of the strains may have varied from their original type, it is not hard to understand why the significance of this reaction was not appreciated. It is very probable that not every observer used a satisfactory medium to obtain this difference.

Buchholz warns of the precautions that must be taken if satisfactory results are to be obtained and states that slight differences in the medium, especially an increase in the agar content, will lead to aberrant results. Fluid media gave unsatisfactory results in his hands. We have also found that the basic medium is of the greatest importance and although some fluid media will demonstrate a certain degree of difference between the "A" and the "B" types, we have been unable to bring out all the differences given with any medium except serum water. Another point that probably led to confusion was the lack of appreciation that a definite degree of resistance to reduction must be possessed by the indicator employed, if all the group differences, which are quantitative only, are to be sharply defined. Buchholz, for instance, found that B. typhosus reduced orcein promptly, whereas, with the indicators we have employed, B. typhosus falls among the non-reducing type. It is not improbable that this dye might show reduction with some of the other groups we have given as non-reducing.

Although with glucose-serum water we have brought out a known reaction with a new medium, it would seem that we had elicited this reaction in a way that gives not only a sharper distinction between strains of human origin but separates the groups of animal origin as well. This has permitted a study of the phenomenon of reduction in correlation with other cultural differences, a correlation hitherto absent.

We have noted above that some irregularity of results has been encountered with glucose-serum water. Although we have not been able to determine all the factors in this irregularity, it is suggestive that nearly all of the trouble has occurred since the use of one Arnold sterilizer, which has been troublesome because of the slowness with which it melts agar. Comparative work has shown that a certain degree of heating is necessary before the medium will give satisfactory results. With a satisfactory sterilizer, heating for from twenty to thirty minutes will usually suffice, but further heating should be given if test-strains do not give sufficiently sharp results. There is some suggestion that if the medium is adequately heated, the differences become greater after the medium is several days to a week old. The serum employed may also be a factor. On the whole, with adequate heating, there is no reason to anticipate any great difficulty in bringing out this reaction with recently isolated cultures. Like all media, some variation in batches is inevitable. Although irregular, intermediate reactions do occur to some extent; they have been encountered nearly always with old stock cultures. These aberrant reactions are due mostly to the tendency of these strains towards variations, and cannot be construed as a criticism of the medium, especially when in some instances this tendency to variation is manifested by a partial or complete reversal of type, in relation not only to this reaction, but to others as well.

The observation of Cole and Onslow.[14] that B. paratyphosus "A" exhibited an ability to rapidly ferment dulcit when grown in their casein-digest medium, should be noted in relation to the observations on avidity we have given. These observations are based on the results obtained with peptone water. The substitution of another medium which would increase the fermentative ability of some of the less active types would lessen or completely obscure the group differences given.

No attempt has been made to note the results obtained by each of the many workers who have employed xylose, dulcit, and arabinose in the study of members of the paratyphoid group. Although differences have been noted, no especial significance had been attributed to these findings. In general, dulcit and arabinose fermentation has been accepted as a characteristic of the group.[10] Ford,[17] however, attributed special significance to the absence of arabinose fermentation with the B. suïs types he studied. He also observed that his "A" and "B" strains differed with xylose, and suggested this as a differential characteristic. This important observation was completely overlooked by subsequent workers. As we noted in our last communication,[2] we were unaware of his results when we presented our findings with xylose.[4] When we had demonstrated this difference, a search of the literature had failed to reveal any report of a similarly significant difference, with the exception of that of Harding and Ostenberg.[18] The latter observed differences in the fermentation of xylose among their paratyphoid types, and although they called attention to the possible differential value of xylose fermentation, they did not conclude that it served to differentiate between the "A" and "B" groups. In most instances they only gave the source and not the type of the strains studied. Evidently the credit of observing the significance of this differential characteristic belongs to Ford and not to Harding and Ostenberg, as implied in the summary given by Jordan, in which the work of Ford is quoted.

Because of the interest of Dr. Welch, a strain of B. icteroides, Sanarelli, obtained from Dr. Ford, was studied to determine whether or not it was a true B. paratyphosus "B" type. By direct agglutination, the strain resembles closely a B. paratyphosus "B." On absorption the strain was found to be unable to absorb the specific agglutinins from either "B" or B. suis sera.

For ease of reference, the cultural characteristics of the main or large sub-groups, thus far separated in the study of the paratyphoid-enteritidis group, are summarized and given in Table XIII. Wherever the observations of other workers are incorporated in the table, or where such more recent results are in agreement with our own, reference is given if the results have not been discussed previously. Where generally accepted fermentation characteristics are inserted to complete the table, no attempt is made to quote those who have reported on these findings. Where additions are made from the observations of others, the degree of avidity cannot be fully noted. In most instances, however, the evidence available points to prompt fermentation.

Possible induced variations are not noted, nor have we discussed this important subject in relation to the findings on avidity already given. The degree of adaptability in relation to different carbohydrates is of the greatest importance, however, in relation to differences in avidity, and also in indicating which fermentative reactions are least subject to variation, and therefore most reliable. The subject has been adequately summarized by Henderson Smith.[19]

TABLE XIII.

MAIN SUB-GROUPS OF PARATYPHOID-ENTERITIDIS GROUP.

Sub-groups.	Glucose-serum Water.[1]	Lead Acetate Agar.	Gas from Glucose.	Acid Production[10] from—																			
				Glucose.	Levulose.	Galactose.	Mannose.	Mannite.	Maltose.	Rhamnose.[4]	Xylose.	Arabinose.[4]	Dulcit.	Sorbite.	Lactose.	Saccharose.	Raffinose.	Erythrite.	Adonite.	Salicin.	Dextrin.[11]	Inulin.	Inosite.[12]
B. typhosus............	+	+	—	+	+	+	+	+	+	—	+ or ±	— or ±	± or —	+	—	—	—	—	—	—	(+)	—	—
B. sanguinarium [6], [7]......	Irreg.	Irreg.	—	+	+	+	...	+	+	±	+ or ±	+	+ or ±	± or —	—	—	—	—	—	—	+	—	—
B. pullorum [6], [7]..........	"	"	+ (—)[3]	+	+	+	...	+	—	+	+ or ±	+	—	±	—	—	—	—	—	—	—	—	—
B. paratyphosus "A".....	+[2]	—[2]	+	+	+	+	+	+	+	+	—	+	±	+	—	—	—	—	—	—	—	—	—
B. abortus equi [8].........	+	—	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—
B. choleræ suis [9].........	+[2]	—[2]	+ (—)[3]	+	+	+	+	+	+[5]	+	+	— or ±	— to +	+	—	—	—	—	—	—	—	—	—
B. paratyphosus "B" [9]....	—[2]	+[2]	+ (—)[3]	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	+ or —
B. enteritidis [9]..........	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—

Notes to Table XIII.

1. With either 1% of Andrade indicator, or 1% of a 1% alcoholic solution of rosolic acid as indicator, + = colored, — = reduction of color.

2. Exceptions noted with old stock strains.

3. Noted occasionally with both stock and recently isolated strains: Dorset,[20] Rettger and Koser,[7] Oette,[2] Wagner,[22] Bock,[23] Ohno,[24] Ten Broeck,[25] Smith and Ten Broeck,[6] Krumwiede and Kohn.[2]

4. For induced variations see Henderson Smith,[19] summary and bibliography; also Krumwiede-and Kohn.[2]

5. Occasional stock strain may show low avidity.

6-7\. Smith and Ten Broeck,[6] Rettger and Koser.[7]

8. Meyer and Boerner.[5]

9. Main representative group. Some strains differ from these in either their cultural or agglutinative characters, or in both. The rodent strains, B. typhi murium and B. danysz, we have studied fall culturally in these groups. There are differences of opinion as to the position of some of the rodent types; see Uhlenhuth and Hübener[10] and Savage[26].

10. The more easily elicited phenomenon of acid production is noted, as differences in gas production tend to be variable with the more complex carbohydrates, and the observation of gas production gives no further differential data. An exception is the vigorous gas production from dulcit by B. abortus equi (Meyer and Boerner),[5] the amount of gas produced being markedly greater than with the other types. Even without eliciting this phenomenon, differentiation is possible according to the data given.

11. The reactions as given for dextrin are those usually quoted. In attempting to repeat the results, however, we obtained varying results according to the samples employed. Thus one sample gave the quoted differences between B. sanguinarium and B. pullorum, but B. typhosus, B. paratyphosus "A" and "B," and B. abortus showed fermentation. With two other samples, neither B. typhosus nor any of the paratyphoid types, including B. sanguinarium, gave a reaction. The results evidently depend upon the constituents of the sample, and without definite knowledge of the constituents of the samples, comparable results are difficult to elicit.

12. Weiss and Rice;[27] see also summary in Muir and Ritchie[28] of observations of English workers on inosite, apparently overlooked by former writers.

SUMMARY.

The tendency to variations, as well as the difference in avidity for carbohydrates, shown by types otherwise similar, has led to contradictory opinions as to the classifying significance of the cultural reactions of members of the paratyphoid-enteritidis group. By correlating the fermentative results, especially in relation to quantitative reductional differences, well-defined groups result, as shown in the tables presented. This grouping correlates host origin and agglutinative differences. B. choleræ suis and B. paratyphosus "B" in this way are separable one from the other, and the cultural differences are parallelled by agglutinin absorption. B. choleræ suis is therefore a distinct group and most of the strains studied have been similar and constitute a definite sub-group. This indicates that B. paratyphosus "B" is essentially a human pathogen and that paratyphoid fever due to this type is normally caused by the transfer of the bacillus from man to man, and that infected swine are not a source of contagion for this disease, either directly or indirectly, through the consumption of infected food.

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STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

VI. The Separation of a Distinct Paratyphoid Group Among Strains of Rodent Origin.*

CHARLES KRUMWIEDE, JR., EUGENIA VALENTINE AND LAWRENCE A. KOHN.

Bacilli of the paratyphoid-enteritidis group have been encountered by many observers in certain types of guinea-pig disease. Some evidence has been advanced that the bacillary infection may be secondary to an infection by a filterable virus (Petrie and O'Brien). Such infections occur rather frequently in epidemic proportions with a very high mortality.

The infected pigs develop pseudo-tuberculous lesions (Smith and Stewart), or the female pigs may develop septic endometritis (Carter). With or without these lesions, the dying pigs develop a septicæmia. In some instances, the organism isolated has been identified as *B. enteritidis* (Gärtner) being culturally identical with this type and agglutinating to the titre of enteritidis serum. More commonly, however, the bacillus isolated has been a paratyphoid type.

Wherry studied a commercial ratticide prepared from this bacillus and gave the bacillus the name, *B. cholerae caviae*. On the recommendation of Smith he changed this to *B. pestis caviae*. Although the studies on the paratyphoid type thus far reported show that the bacillus is a member of the paratyphoid group, its exact position has not been determined, as most of the data is based on direct agglutination alone.

Thus, Smith and Reagh found that their type from pigs showing pseudo-tuberculosis lesions was apparently identical with that of Carter, but both differed agglutinatively from *B. cholerae suis* and from two paratyphoid strains of human origin (gall bladder), presumably "B" types. Wherry likewise found his strains to differ agglutinatively from *B. cholerae suis*. Bofinger found that his strains were agglutinated to the titre of *B. paratyphosus* "B" serum. Kirch concluded that his strains were closely allied to if not identical with *B. typhi murium* and with *B. paratyphosus* "B." He noted some minor variations but assumed these to be due to host adaptations. O'Brien found that his strains were agglutinated to the same titre by both *B. paratyphoid* "B" and *B. suipestifer sera*. He also carried out absorptions of his sera shown schematically as follows:

Strains.	*B. paratyphosus* B Serum absorbed by *B. suipestifer*.	*B. suipestifer* Serum absorbed by *B. paratyphosus* B.
B. paratyphosus "B"	Agglutination.	No agglutination.
B. suipestifer	No agglutination.	Agglutination.
Guinea-pig	No agglutination.	Agglutination.

On this basis, he draws the somewhat remarkable conclusion that "the bacillus (guinea-pig) is indistinguishable by any test at present available from *B. aërtryck* or *B. suipestifer*." Evidently, this conclusion is without foundation as he did not demonstrate that the guinea-pig strains could absorb all the agglutinins from *B. suipestifer* serum. From the cultural side, all have found the bacillus to be very similar to *B. paratyphoid* "B" or to *B. cholerae suis,* although the data are incomplete in relation to fermentative reactions since found to be very essential in differentiating members of the paratyphoid group.

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In infections in mice, at least two types of bacilli are also encountered. One type in its cultural reactions and on direct agglutination is identical with *B. danysz* or *B. enteritidis* (Gärtner). Many stock cultures labeled *B. typhi murium* are this type. Paratyphoid types have also been encountered; most of these strains are very similar to the mouse type, *B. typhi murium,* first isolated by Loeffler. Uhlenhuth and Hübener in their summary consider *B. typhi murium* as a mouse pathogen which is culturally and agglutinatively indistinguishable from *B. paratyphosus* "B." Bainbridge and other English workers class *B. typhi murium* with *B. suipestifer.* The agglutinin absorption results on which their opinion is based are far from conclusive. Thus Bainbridge found that of four stock strains labeled *B. typhi murium,* two were *B. enteritidis* and two were mixtures of two varieties of bacilli. The absorption results of Bock are not above question, as in one of the experiments given the absorption of a *"Typhi murium"* serum by its homologous strain removed the agglutinins for *B. typhi murium* and *B. cholerae suis* but not for paratyphous "B." The absorption results of Levy and Fornet are not convincing. The data available rather indicate that the strains studied have differed and that *B. typhi murium* is not a distinct entity.

The source of the strains we have studied is given in the notes to Table I. No strains have been included which were evidently enteritidis types, either culturally or by direct agglutination. Some of the strains received as *B. typhi murium* have fallen into this group. None of the guinea-pig strains obtained from other workers has been an enteritidis type. From our own pigs we have isolated, however, both the enteritidis and *"pestis-caviae"* types in the same epidemic.

As has been noted in our previous reports, all the paratyphoid strains of mouse and guinea-pig origin, exclusive of the enteritidis types, have been culturally very similar to the *B. paratyphosus* "B" type of human origin. The essential cultural and fermentative characteristics are given in the following table (I.). One suggestive difference, however, has been encountered, although being a group reaction it has little or no practical value in the differentiation of individual strains. That is, the rodent groups show a somewhat lower avidity for xylose than does the *B. paratyphosus* "B" group. This is only appreciably evident with media (peptone or serum water) containing 0.1 per cent. of xylose.

The glucose serum water reaction given in the table is a reduction phenomenon, elicited with a serum water medium containing 0.1 per cent. of glucose and the Andrade indicator. The fermentation of the trace of glucose yields sufficient acid to cause the color of the fuchsin to return, some strains reducing this color (—), other strains not (+). The details of this reaction as well as the use of other dyes as indicators are given in our previous reports.

The only fermentative result that needs comment is that of inosite. All the freshly isolated strains of *B. paratyphosus* "B" we have studied have fermented this substance. As the majority of stock strains also ferment inosite, this suggests the possibility that the stock strains showing low avidity for or inability to ferment it may have originally possessed this characteristic. In the case of the guinea-pig strains, however, we have

TABLE I.

Strains.	Glucose Serum Water.	Lead Acetate Agar.	Gas from Glucose.	Acid Production.												
				Glucose.	Mannite.	Maltose.	Rhamnose.	Xylose.	Arabinose.	Dulcit.	Sorbite.	Lactose.	Saccharose.	Raffinose.	Salicin.	Inosite.
B. paratyphosus "B"	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+ ± or —
B. typhi murium, Nos. 116, 150, 151, 154	—	+[1]	+	+	+	+	+	+[2]	+	+	+	—	—	—	—	+
B. pestis caviæ, Nos. 109, 148, 171, 172, 174, 276, 279, 280	—	+	+	+	+	+	+	+[2]	+	+	+	—	—	—	—	+
B. pestis caviæ, Nos. 108, 110, 117, 146, 147, 173, 278	—	+	+	+	+	+	+	+[2]	+	+	+	—	—	—	—	—
Rabbit No. 107	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—
Cat No. 166	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—

NOTES TO TABLE I.

SYMBOLS—

Glucose serum water, — = pale or colorless, dye reduced.
Lead acetate agar, + = browning of agar.
Acid production, + = prompt fermentation.
± = delayed fermentation.
— = no fermentation.

NOTES—(1) Strain No. 116 reacts more slowly and less vigorously than other strains.
(2) Slight delay noted in some instances, but beginning fermentation usually evident in first twenty-four hours.

SOURCE OF CULTURES—

B. typhi murium, Nos. 116, 150 and 151, stock cultures. History not ascertainable. No. 154, Museum of Natural History, labeled "Johns Hopkins."
B. pestis caviæ, Nos. 108, 109, 110, 146, 147, 148, 171, 172, 173 and 174, from Ferry. See Journ. Path. and Bact., 1914, xviii, 445.
No. 117, isolated by us 1915, in New York stock of pigs.
Nos. 276 and 278, isolated by us in 1917 from breeding stock at Otisville.
Nos. 279 and 280 from Siler.
Rabbit—No. 107. Ferry—see above.
Cat—No 166. Isolated by us 1914.

recovered negative and positive strains (No. 276 and No. 278) from the same outbreak, the strains being tested immediately after isolation.

The results of the direct agglutinations are given in Table II. All the agglutinations have been carried out with broth cultures diluted about one half, depending on the density of the culture, with formalin-salt solution. The final content of formalin was 0.1 per cent., as advised by Dreyer. The results of the direct agglutinations indicate that the rodent strains are different from either *B. paratyphosus* "B" or *B. cholerae suis.* Different "B" sera show differences in the degree of cross agglutination with rodent strains. The goat serum No. 225 has a very low content in group agglutinins, whereas the horse serum No. 684 shows somewhat more cross reaction. In a previous series of tests with another goat serum and with saline suspensions of agar-grown bacilli, an even wider degree of cross reaction was encountered. Possibly a high content in group agglutinins in the "B" serum employed has led previous workers to conclude, on the basis of direct agglutination, that the rodent strains were closely similar to if not identical with *B. paratyphosus* "B."

The very marked mutual cross agglutination with the *Murium* and *Caviae* sera of some members of these groups is strongly suggestive of a close relationship. Some of the rodent strains are agglutinated poorly or not at all by any of the sera indicating the presence of subgroups.

The results of agglutinin absorption are given in Table III. They parallel closely the results of direct agglutination and indicate the presence of two groups among the rodent strains, without regard to host origin; one being separable from the other by the presence or absence of agglutination with the rodent sera available. The mutual cross absorptions by strains Nos. 146 and 154 are complete evidence of the identity of these strains regardless of their host origin. The absorptions of Serum No. 146 show that six strains, two of mouse origin and four of guinea-pig origin, are identical.

Single strain absorptions were carried out with eight other strains, Guinea-pigs Nos. 108, 147, 171, 174, 278, 280, and Rabbit No. 107 and Cat No. 166. Each of these strains showed the capacity of completely absorbing the agglutinins from *B. pestis caviae* Serum No. 146. This demonstrates their identity with the other strains having a like absorptive capacity as given in Table III.

Only strains having the cultural characteristics of the paratyphoid-enteritidis groups have been considered in this work. It is worthy of note, however, that we have encountered with some frequency other types in the terminal septicæmias of rodents. These types belonged to the unclassified group of "intermediates." In some instances these strains could be quickly excluded by their ability to ferment carbohydrates not utilized by members of the paratyphoid-enteritidis group. In other instances the strains were less easily excluded because of delayed fermentation. Among the latter the most common aberrant reaction was a delayed or late fermentation of saccharose.

Summary.—Of fifteen guinea-pig strains studied, thirteen were alike in their agglutination reactions. This apparent identity was further verified by agglutinin absorptions. Two strains from mice and one each from a rabbit and from a cat as shown by agglutinin absorption were identical with this group of thirteen guinea-pig strains. A distinct paratyphoid type or group of bacilli is encountered therefore in spontaneous infections in laboratory animals, especially rodents. Some of the strains from mice and guinea-pigs do not fall into the above group. Others belong ag-

TABLE II.

DIRECT AGGLUTINATIONS[1].

Sera....	“*Caviæ*,” No. 146.						“*Murium*,” No. 154.							*B. Suis.*					“B” Type (G).					“B” Type (H).				
Strains.	1,000	2,000	4,000	7,000	10,000	15,000	1,000	2,000	4,000	7,000	10,000	15,000	20,000	1,000	2,000	4,000	7,000	10,000	1,000	2,000	4,000	7,000	10,000	1,000	4,000	10,000	20,000	40,000
“B” type	+	+	±	×	×	—	+	±	±	±	×	—	—	+	±	×	—	—	+	+	+	+	±	+	+	+	+	±
B. suis..	±	±	×	×	—	—	+	±	±	±	×	—	—	+	+	+	+	+	±	±	×	—	—	+	+	+	±	×
Mouse strains 116	±	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
150	+	+	+	+	±	×	+	+	+	+	+	+	±	+	+	±	×	—	+	±	×	—	—	+	+	+	±	—
151	+	±	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
154	+	+	+	+	±	×	+	+	+	+	+	+	±	+	±	±	×	—	±	±	—	—	—	+	+	±	×	—
Guinea-pig strains. 108	+	+	+	+	±	±	+	+	+	+	+	+	±	±	±	×	×	—	+	±	×	—	—	+	+	±	×	—
109	±	×	×	—	—	—	±	×	×	×	—	—	—	±	×	×	×	—	×	—	—	—	—	+	±	×	—	—
110	+	+	+	+	+	±	+	+	+	+	±	×	×	×	—	—	—	—	—	—	—	—	—	×	—	—	—	—
117	+	+	+	+	+	±	+	+	+	+	+	+	±	+	+	±	×	—	±	±	×	—	—	+	+	±	×	—
146	+	+	+	+	+	±	+	+	+	+	+	±	±	+	±	×	×	—	±	×	—	—	—	+	+	±	×	—
147	+	+	+	+	+	±	+	+	+	+	+	+	±	±	±	×	—	—	±	±	×	—	—	+	+	±	×	—
148	+	+	+	+	+	±	+	+	+	+	+	+	±	±	±	±	×	—	±	±	×	—	—	+	+	±	×	—

Guinea-pig strains.	171	+	+	+	+	+	±	+	+	+	+	±	±	×	×	×	—	—	—	±	±	×	—	—	+	±	±	×	—
	172	+	+	+	+	+	±	+	+	+	+	+	±	×	±	±	×	×	—	×	×	—	—	—	+	+	±	×	—
	173	+	+	+	+	+	±	+	+	+	+	±	×	—	×	—	—	—	—	—	—	—	—	—	×	—	—	—	—
	174	×	—	—	—	—	—	±	×	×	×	—	—	—	×	×	—	—	—	×	—	—	—	—	+	±	—	—	—
	276	+	+	+	+	+	±	+	+	+	+	+	±	±	+	±	×	×	—	+	±	×	—	—	+	+	±	±	—
	278	+	+	+	+	+	±	+	+	+	+	+	±	±	—	—	—	—	—	—	—	—	—	—	+	±	—	—	—
	279	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	×	—	+	+	±	—	—	+	+	+	±	×
	280	+	+	+	+	+	+	+	+	+	+	+	+	±	±	±	×	×	—	±	×	×	—	—	+	±	×	—	—
Rabbit107		+	+	+	+	+	±	+	+	+	+	+	+	±	±	±	×	—	—	±	×	—	—	—	+	+	±	×.	—
Cat 166.		+	+	+	+	+	±	+	+	+	+	+	±	×	×	×	—	—	—	±	×	—	—	—	+	+	×	—	—

Notes to Table II.

[1] None of the strains were agglutinated beyond the range of group action by *B. abortus equinus*, *B. enteritidis* or *B. paratyphosus* "A" sera.
Source of Sera: Nos. 146 and 154, rabbit; *B. suis* and "B" type (G), goat; "B" type (H), horse.
Symbols: + = complete agglutination; ± = marked agglutination; × = slight agglutination; — = no agglutination.

TABLE III.

AGGLUTININ ABSORPTIONS.

Sera	*B. Caviæ*, No. 146.																			*B. Murium*, No. 154.		
Absorbing strain	"B" Type.					No. 110.		No. 117.		No. 276.		No. 279.		No. 150.		No. 154.			No. 146.		
Strains	150	500	1,000	4,000	10,000	150	1,000	150	1,000	150	1,000	150	1,000	150	1,000	150	500	1,000	150	500	1,000
"B" type	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
B. suis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
Mouse strains 116	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
150	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
151	±	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
154	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
Guinea-pig strains 108	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
109	±	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
110	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
117	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
146	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
147	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—

Guinea-pig strains	148	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	171	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	172	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	173	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	174	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	276	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	278	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	279	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
	280	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
Rabbit 107		+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—
Cat 166		+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	×	—	—	—	—	—

NOTE TO TABLE III.

Dilutions higher than 1:1000 not included, to save space. Higher dilutions showed no reaction where lower dilutions were negative, thus excluding the possibility of a pre-zone phenomenon.

glutinatively to the *B. enteritidis* group. Evidently the host origin of a culture is not necessarily an index to its biological position, and the classification of strains according to origin, as *B. typhi murium* or *B. pestis caviae,* is not justified.

None of the rodent strains studied belongs to either the *B. cholerae suis* or *B. paratyphosus* "B" groups. The evidence which has been advanced that some rodent strains fall into these groups is insufficient. The prevalence of the same type of bacillus in spontaneous infections of guinea-pigs in widely separated areas of the country is probably due to the transfer of infected breeding stock.

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A STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE SO-CALLED STREPTOCOCCUS-VIRIDANS GROUP.*

Charles Krumwiede, Jr., and Eugenia L. Valentine.

The increasing recognition of the importance of the nonhemolytic types of streptococci, particularly in focal infections with systemic disease, led us to undertake this study. Such study seemed especially desirable as little information is available as to the immunity relationships of members of this group, most of the investigations having been limited to the determination of biologic characteristics.

The cultures studied included a series known to be pathogenic for man, having been isolated from the blood stream in endocarditis and from other definitely pathologic conditions. For comparison, a series of strains was isolated from the tonsils of healthy individuals. The fundamental criteria used in selecting the cultures were typical streptococcus morphology, especially when they were grown in broth, insolubility in bile or bile salts, and absence of hemolysis as determined by the addition of broth cultures to washed blood cells and the development of greenish color on blood-agar plates. These tests were repeated to eliminate any false negative results. A few strains not in the above category were included for comparative purposes in bringing out certain special points.

All the strains were then studied to determine their fermentative characteristics, inulin, mannite, raffinose, and salicin being used, and to determine their action on washed blood cells—that is, whether they produce methemoglobin or not.[1] The results with respect to fermentation are given in the table.

The determination of the action on washed blood cells did not yield satisfactory results. When all the cultures were tested at one time, varying grades of methemoglobin-production were present, giving an unbroken series of gradations between the two large groups, of sharply positive or definitely negative. Re-tests gave in general like results. Individual false negative results, however, were not infrequent. On the other hand, there was an indication in some instances that an ability to produce methemoglobin which had been dormant, had developed. In some cases a slight discoloration of the supernatant fluid suggested traces of hemolysis as well as methemoglobin-production. With 3 cultures this brownish hemolysis was very distinct, though one of the strains had had no action on washed cells in a test several months previously, and the other two had been described as green-producing types without hemolysis at the time of isolation.

The production of methemoglobin therefore did not sharply divide the nonhemolytic streptococci into two groups. The quantitative character of the results may have been due to the fact that the conditions for the producion of methemoglobin were not satisfactory, especially as false negative results were not uncommon, as stated above. Unless the reaction can be made more uniform and sharper in results, much of its value is lost. At least it cannot be used as the basis for a primary grouping of streptococci.

The agglutination tests were made with broth cultures and immune rabbit sera. In most instances it was not necessary to shake the cultures mechanically if the sediment in the broth was carefully avoided. A varying degree of spontaneous agglutination was encountered. This varied from day to day and in most instances could be overcome by shaking. One culture, Endocarditis 14, which was persistently and strongly self-agglutinating, could not be used even when shaken. The normal content of agglutinins was determined in each rabbit serum used and this value deducted in tabulating the results.

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* Published in Jour. Inf Dis., 1916, 19, p. 760.

[1] Lyall: Jour. Med. Research, 1914, 30, p. 487.

It may be of value to give the method used in producing the agglutinating sera as we have been able by this method to produce sera of high titer with great uniformity. The injections were given intravenously and daily, unless Sunday intervened, according to the following scheme: Suspensions heated to 53° C. were employed at first, and then live cultures. The strains were grown on agar slants with a drop of citrated horse blood on the surface. The dosage averaged as follows in terms of total growth on the agar slant: heated, ¼, ½, ¾, ¾; live, ½, ½, 1. The last dose, one slant, was repeated until the total number of injections had covered two weeks. The rabbits were bled from 4 to 5 days after the last injection.

The agglutination results have been combined in one table, the percentage values of the results being given. There seems to be no need of giving all the reactions at the different dilutions of each serum separately. In the case of one serum the titer could not be determined because of spontaneous agglutination, but its action on the other strains is given because the results offer further evidence for deductions made in discussing results.

The agglutination reactions offered at least no basis for grouping of the strains of pathologic origin, though cross agglutinations were present. The only possible exceptions were the endocarditis strains, Nos. 1, 2, and 3. These, which came to us in this order from Mt. Sinai Hospital, were accompanied unfortunately by no data concerning their origin. However, we feel that they were probably 3 successive isolations from the same case, repeated blood cultures being a common practice at this hospital.

A study of the cross agglutinations yields some interesting data. Serum of a rabbit immunized to Endocarditis Strain 10, as well as sera of such animals immunized to other strains, agglutinated Endocarditis Strain 11 to a slight degree, yet serum of a rabbit immunized to Endocarditis Strain 11 did not agglutinate Endocarditis Strain 10, nor the other apparently related strains. The most striking point is that certain strains were agglutinated to a varying degree by many sera, and this was more noticeable among the normal throat cultures. Furthermore, sera of rabbits immunized to these strains from normal tonsils agglutinated, as a rule, a higher proportion of the tonsillar strains and commonly at much higher dilutions. The sera of rabbits immunized to the tonsillar strains which were agglutinated by sera of animals immunized to the types of pathologic origin, had no action with few exceptions (Endocarditis Strains 7, 9, and 11, and rheumatism, pericarditis, and furuncle strains) on the latter types. Thus serum of an animal immunized to Strain K 2, a strain which was acted upon by most sera, did not agglutinate any of the strains of pathologic origin used in the preparation of those sera. In contrast to this, the three tonsillar strains, K 2, W 2, and M 5, showed a sharp degree of interagglutination. This would suggest a tendency to grouping, if one could explain the fact that these strains were acted upon by other sera when a reverse action was absent.

On the whole, the data seem to us to justify the conclusion that the types pathogenic for man constitute a heterogeneous group. From the practical standpoint, at least, this conclusion seems justified; that is, the possibility of a multiplicity of groups is not excluded by the amount of work we have done. A few of the types from pathologic conditions and many of the tonsillar types tended to possess a common agglutinin-binding content but did not necessarily show a common agglutinogenic content, as they did not cause the production of agglutinins active against other strains unless these strains had given evidence, at least to some extent, of possessing a common agglutinin-binding content for several sera. This deduction seems warranted, though certain factors must be considered in relation to the cross agglutinations. The tonsillar strains showed a greater tendency, numerically, to spontaneous agglutination and a somewhat higher proportion were acted upon by the normal agglutinins present in the rabbit sera. Many strains showed a partial or slight agglutination in a dilution of 1:10. A few strains were agglutinated in dilutions over 1:10, but only partially. These reactions are noted in the table. The majority of these high reactions occurred with the three sera from rabbits injected with the tonsillar strains. In other words the sera of these rabbits had a higher content of normal agglutinins.

The greater tendency, therefore, of the tonsillar strains to be agglutinated spontaneously, and the greater number of these strains agglutinated in the normal serum, probably contributed in producing the results. The instability of the suspension as evidenced by the tendency to spontaneous agglutination would reveal traces of agglutinins otherwise inactive. The fact that the three rabbits used for the tonsillar strains had a higher normal content of agglutinins is probably in part responsible for the great number of cross agglutinations; that is, having a high content of normal agglutinins, they may have responded quantitatively higher in producing group or common agglutinins. This would again be further intensified when they were acting on the unstable suspensions. Altho these factors probably explain the high figures, they do not invalidate the deduction already given.

The agglutination results are in accord with the enormous multiplicity of types which can be differentiated by the determination of fermentative characteristics. On the other hand, there is no correlation between interagglutinations and fermentative reactions, even where the former are very marked.

There is little in the voluminous literature on streptococcus differentiation that is available for comparison with our results. Floyd and Wolbach[2] determined the interagglutination only of cultures representative of cultural subgroups of the streptococcus group as a whole. Kligler[3] also employed agglutination in the attempt to correlate the results of this reaction with fermentative characteristics, studying the streptococcus group as a whole. He used sera prepared against 4 representative strains. Serum of an animal immunized to one endocarditis strain agglutinated only its homologous strain. A serum immunized to a strain from a normal tonsil agglutinated to some extent, strains from endocarditis, 2 from pleurisy, 1 from a lymph node in scarlet fever, and 1 from an antrum slightly. The only striking cross agglutination was with a strain from acute tonsillitis. We feel that the lack of ability of this last strain to cause hemolysis places it in all probability among the nonpathogenic mouth types. If this is so, he has demonstrated a sharp cross reaction similar to our own. The degree of reverse agglutination was not determined in any case.

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[2] Jour. Med. Research, 1914, 24, p. 493.
[3] Jour. Inf. Dis., 1915, 16, p. 327.

TABLE 1.

RESULTS OF THE STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE STREPTOCOCCUS-VIRIDANS GROUP

Strains and Origin.		Inulin.	Mannite.	Raffinose.	Salicin.	Blood.
Endocarditis	1	−	−	−	+	+
	2	−	−	−	+	+
	3	−	−	−	+	+
	4	−	−	−	+	±
	5	+	−	+	..	+
	6	−	+	+	+	+
	7	+	−	+	+	±
	8	+	+	+	+	−
	9	−	−	+	−	+
	10	+	−	−	+	±
	11	−	−	−	−	+
	12	+	−	+	−	+
	13	+	−	+	+	±
	14	+	−	+	+	$\underline{\underline{\pm}}$
	15	−	−	−	+	+
Meningitis	1	−	+	−	+	H^1
	2	−	−	−	−	$\underline{\underline{\pm}}$
Apical Abscess	1	−	−	+	+	+
	2	−	−	−	+	−
	3	−	−	−	+	−
	4	−	−	+	−	+
	5	+	−	+	−	$\underline{\underline{\pm}}$
M. rheumaticus (Beattie)		−	+	+	+	+
Acute rheumatism		−	+	−	+	H^1
Rheumatism and pericarditis		+	−	−	+	±
Arthritis (foot)		−	+	+	+	+
Prostate (arthritis)		−	+	+	+	±
Furuncle (neck)		−	−	+	+	−
Chronic bronchitis		−	−	+	+	H^2
Tonsils, normal individuals (the initial indicates the individual; the number, the strain)	V_3	−	−	+	−	−
	K_1	+	−	+	+	+
	K_2	−	−	+	+	−
	N_1	−	−	+	+	−
	N_2	−	−	+	−	−
	T_1	−	−	−	−	+
	W_1	+	−	+	+	+
	W_2	+	−	+	+	−
	W_3	+	−	+	+	−
	W_4	+	−	+	+	−
	W_5	+	−	+	+	−
	H_1	+	−	−	−	+
	H_4	+	−	−	−	+
	H_5	−	−	+	+	−
	B_2	+	−	+	+	+
	B_3	−	−	−	−	+
	B_4	−	−	+	+	+
	M_2	−	−	+	−	+
	M_5	+	−	+	+	−
	S_3	+	−	+	+	−
	S_4	−	−	+	+	−
	S_5	−	−	−	−	+
	Ta_2	−	−	+	+	+
	Ta_3	+	−	−	−	+
	Ta_4	+	−	−	−	+
Titer of sera		..	.	..	..	..

TABLE 1—*Continued*

RESULTS OF THE STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE STREPTOCOCCUS-VIRIDANS GROUP

Agglutinating Sera									
Endocarditis No. 1	Endocarditis No. 2	Endocarditis No. 6	Endocarditis No. 10	Endocarditis No. 11	Endocarditis No. 14	Apical Abscess No. 2	Tonsil W_2	Tonsil K_2	Tonsil M_5
100	100	0	0	0	—	0	0	0	0
50	100	0	0	0	—	0	0	0	0
55	100	0	0	0	—	0	0	0	0
0	0	0	0	0	+	0	0	0	0
0	0	0	1	0	+	0	0	0	0
0	0	100	0	0	—	0	0	0	0
1	0	1	0	0	—	0	2	2*	10
0	0	0	0	0	—	0	0§	0*	0†
1	0	0	1	0	+	0	33	10	33
1	0	0	100	0	—	0	0	0	0
1	0	0	12	100	+	0	10*	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
S	S	S	S	S	S	S	S	S	S
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	100	0	0	0
0	0*	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
3	5	0	2	0	—	0	12	5	33
0	0	0	1	0	—	0	0	0	0
6	0	0	0	1	+	0	0*	0*	0†
6	20	1	1	0	—	0	5	0	7
1	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
1	0	0	0	0	+	0	50§	66*	33‡
25	50*	0	2	1	+	0	150	100	100
20	32	0	0	0	—	0	20	7	66
1	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
1	5	0	0	0	+	0	25*	1*	0
20	50*	0	1	1	+	0	100	8	10
10	32	0	3	0	+	0	100	33	66
0	0	0	3	0	+	0	12*	2*	10†
5	20	0	3	0	+	0	12	10*	10
0	0	0	0	1	—	0	0	0	0
1	0	0	0	0	—	0	0	0	0
1	1	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	12	2	10
1	1	0	0	0	—	0	12	3*	17
20	10	0	0	0	—	0	12	3	17
0	0	0	1	1	+	0	0*	0*	2*
1	1	1	1	0	+	0	12	1*	100
10	5	0	0	0	+	0	2†	2	10
1	0	0	0	0	+	0	0†	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
400	200	800	600	400	?	180	400	600	600

Explanation of Table.

Blood.—In this column + means definite methemoglobin-production; ±, moderate methemoglobin-production; ⩲, slight or doubtful methemoglobin-production; —, no change; H, hemolysis (1) reddish brown, (2) deep garnet.

Agglutination.—To economize space and to make the results directly comparable and easy of interpretation, percentage values are given. The actual results were first reduced to numeric values according to the dilution results. Thus a complete reaction at 1:200 was given a value of 200. A half complete reaction was given a numeric value of half the dilution, and so on. The latter procedure was followed only in low dilutions, exceptionally in higher dilutions where a partial reaction persisted. In these instances the numeric values may be too high, but for our purpose this is preferable to underestimation. The figures so obtained were then converted into percentage values using the figure 100 for the homologous strains. The highest dilution (titer of sera) giving a complete reaction with the homologous strain, is given. In the case of the serum immunized to Endocarditis 14, the titer not being determinable (S = spontaneous agglutination), the presence or absence of agglutination is noted only with the symbols + and —. Action of normal agglutinins in dilutions over 1:10 are noted as follows: (*) = 1:50, (†) = 1:100, (§) = 1:200, referring to the highest dilution showing any agglutination. In one instance (‡) traces were present up to 1:600.

SUMMARY

Streptococci of the "viridans" type isolated from pathologic conditions apparently constitute a heterogeneous group. Saprophytic types may show some relationship one to the other, but practically none to the pathogenic type. Therapy, serum or vaccine, to elicit specific results is therefore possible only with autogenous strains.

A TRIPLE-SUGAR MODIFICATION OF THE RUSSELL DOUBLE-SUGAR MEDIUM.*

Charles Krumwiede, Jr., and Lawrence A. Kohn.

In the search for paratyphoid bacilli in plates made from feces or other material, there are found many organisms which give typical paratyphoid colonies on the usual media, but ferment lactose slowly or not at all. This may be a source of great annoyance. Savage and Read,[1] in the isolation of Gaertner bacilli from rats, fished the colonies which did not ferment lactose on the plates into fermentation tubes of broth containing 0.3 per cent. each of lactose, saccharose, and salicin. The tubes were held two days, and all organisms producing gas were excluded.

In this country the double-sugar medium devised by Russell [2] has found universal favor in identifying members of the colon-typhoid group. As we have previously reported,[3] sharper reactions are obtained when Andrade's indicator is substituted for the original litmus; moreover, with this modification the tubes can be read by artificial light, and there is no decolorization of the butt with an ambiguous reaction. Unaware of Savage and Read's procedure, we found that many "intermediates," especially those encountered in feces, gave a typical paratyphoid Russell reaction, although some of them proved to be slow lactose-fermenters. On studying these organisms, and also the non-lactose-fermenting strains of similar origin, we were impressed by the fact that, growing aerobically, nearly all fermented saccharose more vigorously than lactose. We accordingly attempted to utilize this fact by adding one per cent of the former sugar to Russell's medium.

A number of "intermediates" were isolated from feces, and tested immediately. Of eighty-five strains tested, forty giving a typical paratyphoid reaction on Russell's double-sugar medium could be immediately excluded on triple-sugar medium. An additional eighteen of these eighty-five gave a faint or delayed color to the slant on Russell's medium, but an intense and prompt color on the triple-sugar slant. The remaining twenty-seven remained typical on both media. In short, forty-seven per cent could be immediately excluded on triple-sugar medium, and an additional twenty-one per cent gave sharper reactions.

For the preparation of the triple-sugar medium we use a stock agar, the basis for our brilliant green medium,[3] containing 1.5 per cent. agar, .3 per cent. meat extract, 1 per cent. peptone, and .5 per cent. salt. This is adjusted roughly at slightly alkaline to litmus (or .6 per cent. acid to phenolphthalein, by hot titration) and finished in the usual way. At the time of use, one per cent. of lactose, one per cent. of saccharose, and one-tenth per cent. glucose are added to the melted agar. These are conveniently added from a stock solution containing 20 per cent. lactose, 20 per cent. saccharose, and 2 per cent. glucose, sterilized intermittently. Five cubic centimeters of this solution added to each one hundred cubic centimeters of agar gives the required concentrations. One per cent. of the Andrade indicator is then added, and the reaction adjusted. The agar should be red when hot, and should completely lose color on cooling a small portion under the tap. If it is not red when warm, it is too alkaline; if it fails to decolorize on cooling, it is acid, and it is corrected accordingly by adding small amounts of normal acid or alkali solution. It is then tubed from a sterile covered funnel, preferably in narrow tubes of a diameter approximating one centimeter inside diameter, and steamed for twenty minutes only, to kill chance contaminants. The tubes are then slanted, leaving a generous butt for stab inoculation, and incubated over-night for sterility. Each preparation is tested before use with known strains of dysentery, typhoid, paratyphoid, and colon bacilli.

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* Published in Jour. Med. Res., 1917, 37, p. 225.

Occasional atypical reactions are encountered. It appears that the colorless slant is brought about in part, at least, by reduction of the color. With feeble growth of either B. typhosus or B. paratyphosus "A," especially where the medium is fresh and moist and the organism extremely motile, the slant may be slightly pink. This error may be avoided by setting the reaction from 0.2 per cent to 0.5 per cent more alkaline, but in this case the feebler fermenting dysentery types may produce only a slight tinge of color in the butt. Re-incubation will clear the slant if false reddening occurs. It is well, therefore, to scrutinize carefully all slightly pink slants, and discard only those in which the character of the growth, or excessive gas production, are exclusive. Others should be re-incubated, as well as those tubes in which no color has developed, to verify acid production. The rarer saccharose-fermenting dysentery type (Strong) may give a red slant and red butt without gas. Morphological examination of a tube with this reaction may allow immediate exclusion, as this reaction is commonly due to cocci. Whether these atypical reactions occur with Russell's original medium, or are due to the modifications we have made, we cannot say. They are so infrequent and are so easily checked that the other advantages of the modified medium far outweigh them.

For tentative differentiation between typhoid and dysentery bacilli, both of which give acid butts, a trace of growth may be added to a drop of broth with the usual cover-slip method.

We have used this medium for some time with satisfactory results, and it is reported at this time because of the greater possibility of encountering paratyphoid bacilli.

SUMMARY.

When Andrade's indicator is substituted for litmus in the Russell medium, sharper results are obtained. Many "intermediates" have a greater avidity for saccharose than for lactose, when grown aerobically. By the use of the triple-sugar medium described, many of these types, which give a doubtful or typical paratyphoid reaction on Russell's medium, are promptly excluded.

REFERENCES.

1. Savage and Read. Journ. Hygiene, 1913, xiii, 343.
2. Russell. Journ. Med. Research, 1911, xxv, 217.
3. Krumwiede, Pratt, and McWilliams. Journ. Inf. Dis., 1916, xviii, 1.

FURTHER EXPERIENCES WITH BRILLIANT GREEN AGAR AND OTHER PLATING MEDIUMS FOR THE ISOLATION OF TYPHOID AND PARATYPHOID BACILLI FROM FECES.*

Charles Krumwiede, Jr., Lawrence A. Kohn, Anne G. Kuttner, and Elsie L. Schumm

Since we described a method of preparing a brilliant green agar plating medium for the isolation of typhoid and paratyphoid bacilli from feces.[1] the number of specimens examined has reached a total of over 7,000. It seems advisable, therefore, to present the results obtained and also some slight modifications of the methods employed, as dictated by the experience gained in handling this large amount of material. Since our report other observers have suggested various plating mediums for fecal examinations and these have been tried sufficiently to determine their practical value. Our opinion as to their merits, as based on our experience, is also given.

As we have modified the preparation and standardization of our medium somewhat it seems advisable to describe the medium and the methods as now employed. This will also avoid the inconvenience of reference to our former report.

Preparation of Medium.—The composition of the agar base is as follows:

Liebig's Extract of Beef	3 gm.
Peptone	10 gm.
NaCl	5 gm.
Agar	15 gm.
Water	1 liter

The agar is dissolved in half the water by autoclave, the other ingredients in the remainder of the water by heating on a gas stove. After complete solution the two halves are mixed and the reaction adjusted by the addition of alkali. The final reaction desired is the neutral point of the Andrade indicator. This indicator is prepared by adding 16 c.c. of N/1 NaOH solution to 100 c.c. of 0.5 per cent. aqueous solution of acid fuchsin. The alkali changes the red of the fuchsin to orange or yellow. Mediums adjusted to the neutral point of this indicator are pink when hot and colorless when cold, which corresponds to between 0.6 per cent. and 0.7 per cent. acid to phenolphthalein (hot titration). The reaction of the agar may be adjusted to the phenolphthalein reaction given or directly adjusted to the Andrade indicator by adding 1 per cent. of the indicator to a small measured volume and determining how much alkali is needed to bring it to the point desired, namely, pink when hot and colorless when cold. After adding the appropriate amount of alkali, thus determined, to the bulk of the medium, the latter should be retested and readjusted if necessary. The Andrade indicator adjusts itself somewhat slowly to changes in reaction; a little time therefore should be allowed to elapse between each addition of soda. We have found that with Fairchild's peptone and Liebig's extract the routine addition of 8 c.c. of normal soda per liter will give a satisfactory end reaction. This will vary depending upon the brand of peptone and extract employed, but once the necessary amount of soda is determined, the reaction can be closely approximated by employing this amount in succeeding batches of medium, avoiding in this way the necessity of titration. After adjusting the reaction, the agar medium is cooled and beaten egg white added. It is then autoclaved for one-half hour at 15 pounds' pressure and filtered. After filtration the entire preparation is mixed thoroughly to insure uniformity of the whole product, bottled 100 c.c. to a bottle and autoclaved for thirty minutes at 15 lbs. pressure.

At the time of use the needed number of bottles are melted and to each 100 c.c. of agar is added 1 c.c. of the Andrade indicator, 1 per cent. of lactose, 0.1 per cent. of glucose and finally the appropriate amount of brilliant green. For the addition of the carbohydrates it is most convenient to prepare a stock solution in distilled water, containing 20 per cent. of the lactose and 2 per cent. of glucose, sterilizing the solution by steaming in the Arnold sterilizer. Five c.c. of this solution added to 100 c.c. of agar will give approximately the percentages required. The brilliant green is added from a stock 0.1 per cent. solution in distilled water. Two concentrations of brilliant green are employed, the appropriate amounts being determined by preliminary standardization (see below under standardization). The medium is mixed, poured into plates so as to give a rather thick layer allowing 6 plates for every 100 c.c. of medium. The finished plates must be dry and this is best assured by using porous earthenware covers. With these, the medium can be covered as quickly as poured without the formation of water of condensation.

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* Published in Jour. Inf. Dis., 1918, 23, p. 275.

[1] Krumwiede, C., Jr., Pratt, J. S., and McWilliams, H. I.: Jour. Infect. Dis., 1916, 18, p. 1.

Standardization.—This is the difficult step and if not successfully carried out, much or all of the value of the medium is lost. In our previous report we advised the use of the Rawlings strain of B. typhosus for standardization. This strain, however, has fluctuated so much in its susceptibility to brilliant green that its use has been discontinued. Freshly isolated cultures have given somewhat better results but positive stools, if obtainable, are by far the more reliable material for this purpose.

The stock brilliant green solution will keep for about a month. A fresh solution therefore should be employed in standardizing a new batch of agar and the batch of medium should be as large as possible so that the number of standardization tests will be reduced to a minimum.

As each new batch of medium is received plates are poured using 3 dilutions of the dye, namely, 1 : 200,000; 1 : 330,000, and 1 : 500,000. For convenience these are expressed in terms of the fractions of a c.c. of a 0.1 per cent. solution of brilliant green added to a 100 c.c. of agar; the amounts are, respectively, 0.5, 0.3, and 0.2 c.c.. The plates are then inoculated with equal amounts of material, spreading it by means of a spatulum. A non-restraining medium such as Endo is used as a control. The best material for standardization is found in positive stools from typhoid carriers or cases. If obtainable, several fecal specimens should be used to compensate for the variability of the fecal flora, slight variations in resistance of different strains of B. typhosus, and to average the variables due to technic. If only one positive stool is available this should be plated in duplicate or used to inoculate suspensions of 2 or 3 normal stools which are then used for the standardization tests. The suspensions should be comparatively light so that the resulting plates will have discrete colonies, otherwise comparisons by count or estimate will be difficult or impossible.

The stools should be freshly collected as the persistent types in older stools tend to be the dye-resistant types and are therefore not satisfactory as a measure of the restraining action of the dye on the average fecal flora. If positive stools are not available broth cultures of recently isolated typhoid strains may be added to suspensions of fresh normal stools and the plates inoculated. The addition of a loop to 0.1 c.c. of an 18-hour broth culture to 10 c.c. of stool suspension of moderate density will give a well balanced mixture of B. typhousus and fecal bacteria. Pure cultures of B. coli should not be used to determine the restraining action of the dye. The degree of restraint of the average fecal flora is the fact wanted.

After incubation the plates are inspected and, based on the results, selections are made of the dilutions to be used. Two dilutions are chosen: first, the greatest amount of dye which has little effect on the number and size of the typhoid colonies, but which shows a marked restraint of the fecal flora; second, a greater amount which shows a moderate reduction in the number and size of the typhoid colonies, and usually a still greater reduction of the fecal flora. Slightly greater concentrations of dye are used in practice than would seem to be indicated by the standardization results as the inoculation will be heavier, thus reducing the activity of the dye to some extent. With most batches of mediums and with the sample of dye we employ the 2 optimum dilutions average 0.2-0.3 c.c., respectively, of a 0.1 per cent. solution of the dye to 100 c.c. of agar. (See brands of dye.) The reasons and advantages of two concentrations of the dye are given later.

Interpretation of the results of standardization tests and the selection of the optimum dilutions are most easily and accurately determined by a graphic representation of the results. Averages are taken of the figures recorded for the different specimens on the Endo medium and on the dye agar plates according to concentrations tried and the following data charted: the average percentage reduction on the dye plates of (a) colonies of fecal bacteria other than typhoid, (b) typhoid colonies, and (c) reduction in size of typhoid colonies using 1 mm. as the standard. In the accompanying representative charts (Charts 1, 2, 3, and 4) the results with fecal typhoid and culture typhoid strains are given separately for comparison. The selections that should be made for routine use are indicated by a heavy vertical arrow. The curves show clearly the basis of selection. Charts 1 and 2 are representative of the general limits of fluctuation with different batches of agar with the dye we have used in our routine work.

The two dilutions selected according to this method serve well in routine work for the isolation of both B. typhosus and B. paratyphosus. When the latter is the only type to be sought, one strong concentration may be employed alone, as this type is much more resistant to the dye than B. typhosus. An advantage of using stronger concentrations in this case would be the greater degree of restraint of the accompanying fecal flora. With a standardization result indicating that 0.2 c.c. and 0.3 c.c. are satisfactory for the isolation of B. typhosus, from 0.35-0.5 c.c. can be used for B. paratyphosus. During an outbreak of paratyphoid fever, where one strain is presumably common to all the cases and carriers, the medium can be directly standardized, using a positive stool or a freshly isolated strain as soon as one is obtained. Because of the greater resistance of B. paratyphosus, wider limits of error are allowable than in standardizing for B. typhosus, and freshly isolated cultures give very satisfactory results. We have recently employed this method with marked success in the case of a regiment infected with B. paratyphosus A.[2]

Adaptability of Various Brands of Dyes.—Various samples of dyes have been tried. Of these the Bayer, Grübler and Hoechst brands have given successful results and the dilution range of differential action was closely alike. Of the other samples, bearing no name but probably of German manufacture, some have been fairly good. Examples are given in Charts 3 and 4. Although good differential action was obtained with both samples a considerable concentration was needed, indicating that there were inactive impurities present. Although we have not personally encountered dye samples which, even when very dilute, inhibited B. typhosus as well as the fecal flora, we have had a report of such an experience. The results would seem to indicate the presence of highly toxic impurity.

Colony Characteristics.—On the medium, after 18 hours' incubation, the typhoid colonies will be found to be very characteristic. They are of good size (1-1.5 mm.); the glucose not only enhances their growth but is also the main cause of their characteristic appearance. Viewed through the plate against a dark background the light passing obliquely through the agar they have a peculiar striated flaky appearance. With artificial light and a hand lens under the same conditions they have the appearance of a coarse wool fabric. They may take on a faint mauve tinge from fermentation of the trace of glucose, which accentuates somewhat their characteristic appearance. The larger colonies may resemble the paratyphoid B type. Colonies of this organism tend to be larger, heavier and more opaque. They are often tinted a delicate green, and the markings are less distinct, though still evident at the periphery. The paratyphoid A colonies resemble the typhoid colony more nearly than that of the paratyphoid B, but we have frequently observed an extremely flattened, slightly tinted colony whose edges melt into the agar. The markings of the colonies of all three organisms are usually more prominent on the stronger dye plates. The only organisms which simulate the typhoid colony are certain partially restrained colon types which may develop on weak dye plates. They present exaggerated markings, with distinct cross-bar striations, and they are usually completely excluded on the stronger dye plates. These organisms frequently agglutinate spontaneously in the saline drop (see below). Some of the dye-resistant "intermediates" develop paratyphoid-like colonies.

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[2] Krumwiede, C., Jr.: Jour. Infect. Dis., 1917, 21, p. 141.

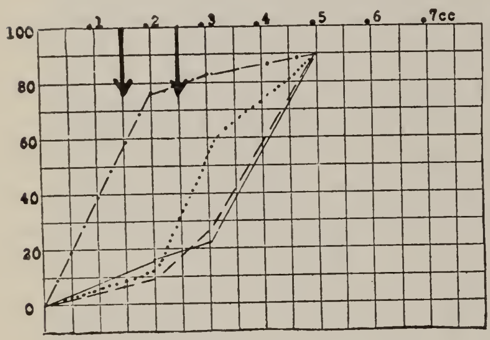

Chart 1.

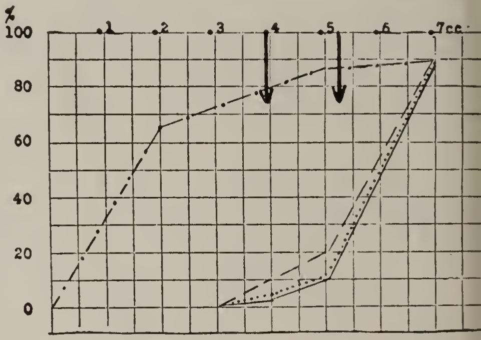

Chart 3.

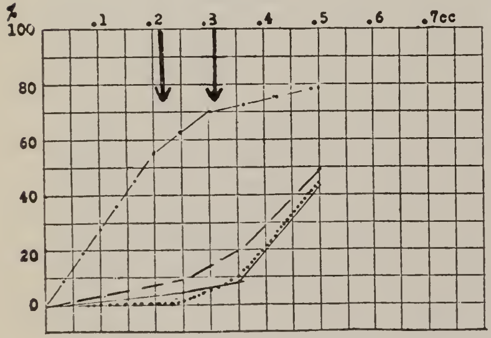

Chart 2.

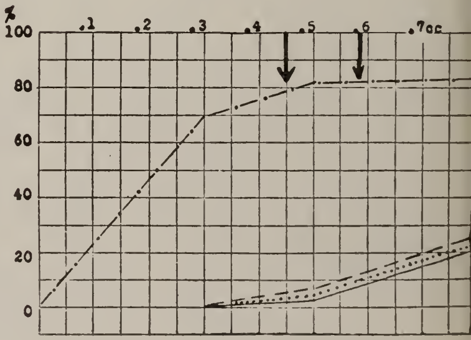

Chart 4.

Explanation of charts: —.—.—.— = Fecal types; ———————— = Fecal typhoid; .... = Culture typhoid; — — — — — — — Size of typhoid colonies.
Charts 1 and 2.—Standardization test. Bayer dye.
Charts 3 and 4.—Standardization test. Dye? See under Brands of Dye.

With no known change in the mode of preparation an occasional batch of agar will develop very large typhoid colonies. These luxuriant colonies tend to be much less characteristic in their markings. The tendency to the development of this type should be noted when standardization tests are made so that such colonies will not be overlooked in using the medium subsequently in routine work. With an agar of this kind slight inhibition by the dye will result in more typical colonies apparently due to a lessening of the density of the colony, thus making the markings more visible. It is possible even with the average batch of agar that the dye, exerting a very slight influence, contributes somewhat to the development of the typical colony.

The colonies of the fecal types which develop take on a moderate pink color if they ferment lactose, but the color change is not marked. Even without a sharp color change, they are so different from the typhoid or paratyphoid colony that the practiced eye passes over them without hesitation. This lack of sharp color change may seem a decided disadvantage to bacteriologists accustomed to the sharp color differences of Endo and similar mediums, on which the typhoid-paratyphoid colonies are presumably those showing no change of indicator. We have approached the problem, however, from the standpoint of giving the colonies of these types positive characteristics, rather than relying on a negative characteristic, inability to ferment lactose. In this regard, the medium is unique, and, to our mind, the more logical way of attacking the problem of evolving a still more satisfactory medium.

Method of Inoculation.—To obtain discrete colonies various methods of inoculation are advised, as for instance the use of successive dilutions of an initial fecal suspension. Such a method takes time and glassware and is not very expedient in handling large numbers of specimens. With some practice one can obtain very satisfactory results by diluting the suspension to a density which experience has shown will yield sufficiently abundant but discrete colonies. This density corresponds roughly to a suspension of 1 part of solid feces to 15 parts of fluid. We dilute fluid feces or emulsify solid feces to this density using peptone water and allow the suspensions to stand 15-30 minutes to allow sedimentation of the particles and then inoculate the plates from the surface of the suspension. To save time inoculation is done with a double loop so that one or both may be emptied on the plate. For each specimen two series of plates are employed each consisting of a weak and a strong dye agar and an Endo plate. The fecal suspension is inoculated only on the dye plates, 1 loop being placed on each of the first series and 2 loops on each of the other series. The material is then spread with a bent nichrome wire, rubbing out the material on the weak plate, then on the strong plate and then passing to the Endo plate, the material adhering to the wire sufficing to inoculate this plate. The second series is then streaked in the same fashion. The stronger dye-plate in each series receives in this way the heaviest inoculation. The spreader is only sterilized between specimens.

Nichrome wire is a cheap and convenient substitute for platinum and a very satisfactory spreader is made by bending the wire to an angle of 120° and bending up the tip to avoid tearing the plates. It does not fuse well in glass and the shank is best bent in a zigzag fashion and pushed into narrow bore tubing with thick wall. Owing to its greater rigidity a finer gage of nichrome wire can be used than in the case of platinum, thus compensating for the slower cooling of the former. If two spreaders are used, one cools while the other is in use. We have found the wire spreader more convenient than glass rods which cool very slowly if sterilized by heat while inoculating, and are inconvenient to wash and sterilize in bundles especially when the number needed is large.

Identification.—Although the preceding gives the details of the medium it seems well to describe again the following methods because of their expediency and reliability in mass work. Preliminary identification of the colonies is done by means of the slide agglutination method using highly potent serum. A drop of highly immune serum in low dilution is placed on a slide, and beside it one of salt solution. The suspicious colony is picked off with a small loop of fine wire, and a sufficient amount rubbed off in the salt solution to give a slight clouding. Some of the growth remaining on the loop is then rubbed off similarly in the serum drop.

An immediate clumping, visible to the naked eye is a positive result; any spontaneous agglutination will be noted in the control. When the colony is suspicious of paratyphoid, 3 drops, salt solution, A and B serum, may be used, a portion of the colony being rubbed in the salt solution and one serum, and another portion in the second serum, with intermediate sterilization of the loop. It is essential, for this purpose, that the serum be of high titer, as a slow reaction is time-consuming and permits drying of the drop. In general, we prefer an older serum of original high potency, which has lost not only in total titer for its homologous type, but also in content of group and normal agglutinins. Under these conditions, the dilutions used may be as low as 1 : 50 to 1 : 100. With a known and tested serum, the results are very reliable; with an unknown serum, serious error may result because of group reaction. Occasionally a slight delay in clumping, due to the prezone phenomenon, will be noticed, but there is usually some indication of a reaction in the serum, and dilution with an additional loop or two of salt solution induces prompt and marked clumping. We have noted increased agglutinability in colonies on dye plates as compared with those on Endo. This is apparently due not to the dye alone but to the glucose as well; in any case, it is no disadvantage. Inagglutinable strains, showing no reaction in the concentrations of serum employed, are rarely isolated from feces, but a characteristic colony, non-agglutinating, should not be overlooked. In emergencies, with known serums and with trained workers, the macroscopic slide agglutination could be considered final for all promptly positive cases with little likelihood of error. The doubtful slower reactions, usually few in number, would have to be verified by isolation. A negative slide agglutination is usually accepted as a final negative result, but we fish some suspicious colonies occasionally as a control on the serum. Positive results are checked by fishing to the Russell medium or better a modified Russell containing 1 per cent. of saccharose as well as 1 per cent. of lactose and 0.1 per cent. of glucose.[3] Paratyphoid-like types which may give some reaction with the paratyphoid serums are more surely excluded in this way, as many of these types ferment saccharose but attack lactose only slightly or not at all. If the reaction on the Russell medium is typical the results of the slide agglutination are verified by using the growth for macroscopic agglutination. If the serums used are of sufficient titer and the range of group and normal agglutinins is known, this suffices for all practical purposes. If the serums are not altogether satisfactory false positive results are likely to be encountered in examining for B paratyphosus and the results are then best verified by determination of the cultural and fermentative characteristics.

If the search is to include the paratyphoid types causing food poisoning (B. cholerae suis and B. enteritidis) fishing of the suspicious colonies becomes necessary as well as the full determination of the cultural and fermentative characteristics and final identification by agglutination with the appropriate serums and possibly agglutinin absorption.

There is one source of error in the use of brilliant green agar which is common to all selective mediums. In fishing a suspected colony, impure cultures may be obtained, as fecal bacteria which have been inhibited are capable of multiplication when transferred to a favorable medium. This danger should be kept in mind, and suitable precautions taken. In fishing, it is well to avoid touching anything but the apex of the colony. It is well to restreak on an Endo plate if there are very few positive colonies in a crowded area and it is questionable whether a pure culture can be obtained.

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[3] Krumwiede, C., Jr., and Kohn, L. A.: Jour. Med. Research, 1917, 37, p. 225.

Results Obtained with the Brilliant Green Medium.—Table 1 gives the numerical results in the isolation of B. typhosus from feces.

TABLE 1.

FECAL EXAMINATION FOR B. TYPHOSUS.

Source.	Totals.	Endo Positive.	Dye-Agar Positive.	Percentage of Endo Positives Compared with Total Positives.
All sources	7,111	920	1,235	74.5
From carriers	594	502	564	89
All sources exclusive of carriers	6,517	418	671	62

As one would expect, the chronic carriers give the least increase in positive results as their feces usually contain a relative abundance of typhoid bacilli allowing of easy isolation with any nonrestraining medium. The fewer the bacilli in proportion to other fecal types the greater should be the expected increase of positive results with the dye-medium. This expectation is realized and shown in the results in Table 2.

TABLE 2.

SPECIMENS FOR DIAGNOSIS FROM SUSPECTED CASES OF TYPHOID FEVER.

Number.	Endo Positive.	Dye-Agar Positive.	Percentage of Endo Positives Compared with Total Positives.
115	18	45	40

Table 3 is of interest in this connection. The examinations were carried out in making a sanitary survey of an insane asylum because of the development of cases of typhoid fever. Nearly all of the positive results were obtained from patients found to be suffering from the disease. Because of their mental state the existence of the disease was unsuspected in some of the patients till positive fecal reports led to routine temperature taking. A rise of temperature was frequently the only indication of the presence of the disease.

TABLE 3.

SPECIMENS, STATE INSANE ASYLUM.

Number.	Endo Positive.	Dye-Agar Positive.	Percentage of Endo Positives Compared with Total Positives.
847	30	46	65

Although the figures indicate the numerical increase of positive results on the dye-agar they do not indicate the greater ease of isolation on this medium in cases in which the Endo medium is positive as well. With a dye-sensitive fecal flora as encountered in the average feces, the suppression of these types on the more heavily inoculated dye-plates results in the development of many more typhoid colonies as compared with the Endo plates or may result in the development of typhoid colonies alone. The contrast between the Endo and dye-plates in the actual number of typhoid colonies and the comparative number in relation to the total number of colonies is usually very striking.

We have continued the use of the Endo medium mainly to obtain comparative data and to some extent as a check on the use of the brilliant green medium because of a frequent change of personnel among those carrying out the actual examinations. With sufficient experience it could be omitted as we have encountered positive results on Endo with negative results on the brilliant green agar in only two instances. In one this seemed to be purely accidental; in the other the dye-plates were overgrown by B. aerogenes types, the feces being unusually rich in this dye resistant variety.

Two concentrations of dye are used for the following reasons: Brilliant green is an exceedingly sensitive colloidal restraining agent and its differential action is quantitative in nature, being elicited only at certain dilutions. The activity of the dye is not necessarily the same in different batches of medium apparently prepared in an identical manner. The restraining action is elicited in higher dilutions in a clear agar than in a cloudy one, and any foreign matter, for example, a trace of cotton in the agar, will reduce the activity of the dye. For this reason the restraining action will vary with the amount of fecal protein inoculated. Furthermore, the fecal flora of different specimens varies somewhat in dye sensitiveness. Strains of B. typhosus likewise vary slightly in their resistance. Two dilutions compensate for these variables and for any slight miscalculation in the selection of the dilutions as based on the standardization test. The stronger dye-plate has one advantage that, although there be a reduction in the number of typhoid colonies developing, the greater restraint of the fecal flora may bring them into greater relief. Table 4 gives the comparative results with the two strengths of dye: it does not show, however, the greater preponderance of B. typhosus on one or the other concentration, which is frequently encountered in examining the plates from an individual specimen. This must be considered in interpreting the results given, especially the greater number of positive results with the stronger concentration of dye.

TABLE 4.

FECAL EXAMINATION FOR B. TYPHOSUS: COMPARATIVE RESULTS ACCORDING TO STRENGTH OF DYE.

Positive on weak dye-agar only	26
Positive on strong dye-agar only	81
Positive on both weak and strong dye-agar	1,128
Total positive on dye-agar	1,235
Total positive on Endo	920

The results in the isolation of B. paratyphosus (Tables 5 and 6) have been somewhat better than in the isolation of B. typhosus.

TABLE 5.

FECAL EXAMINATIONS FOR B. PARATYPHOSUS A.

Number.	Endo Positive.	Dye-Agar Positive.*	Percentage of Endo Positives Compared with Total Positives.
856	30	60	50

*Majority of results based on use of one strength, 0.35-0.5 c c of a 0.1% solution of dye to 100 c c of agar.

TABLE 6.

B. PARATYPHOSUS B.

Number.	Endo Positive.	Dye-Agar Positive.	Percentage of Endo Positives Compared with Total Positives
(11)*	5	11	45

*Encountered while examining for B. typhosus.

On the whole the results obtained have equaled those originally reported as based on only a relatively small series of examinations.

In our original communication,[1] we recommended Kendall's modification of Endo's medium because of the convenience of using the same agar stock for both this and the dye-agar. We have found, however, that although Kendall's modification gives excellent differentiation for the more vigorous lactose fermenters, a great many colonies which were absolutely colorless on this medium, ferment lactose rapidly when transferred to Russell's double sugar medium. We have tried numerous other modifications of Endo and have found the one recently recommended by Robinson and Rettger[4] to be the most satisfactory, giving as it does the sharpest color to the lactose fermenting colonies with the least diffusion. We have changed their method slightly to avoid adding the indicator till the medium is to be used, as there is always the danger of the reduced color returning, especially if large batches of mediums are prepared at one time.

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[4] Jour. Med. Research, 1916, 34, p. 363.

The medium is prepared as follows: Twenty-five gm. of agar, 10 gm. of peptone, and 5 gm. of meat extract to a liter of water, are dissolved by heating on a gas stove. The ingredients are made neutral to litmus by the addition of 10 per cent. solution of sodium carbonate. An additional 10 cc. of sodium carbonate is added per liter. The medium is cooled to 45 C. and one egg per liter added. Autoclave for 30 minutes at 15 lbs. pressure. Filter and add 1 per cent. lactose and bring to a boil to dissolve the sugar. Finally add another 10 cc. of 10 per cent. solution of sodium carbonate per liter, the reaction should now be —0.3 to phenolphthalein (hot titration). The medium is bottled in 100 cc. amounts and autoclaved for 10 minutes at 10 lbs. pressure. To the melted agar just before use, add 0.5 cc. of a saturated alcoholic solution of fuchsin and 1 cc. of a 10 per cent. solution of amorphous sodium bisulphate.

If, due to heavy seeding, diffusion occurs, typhoid colonies may nevertheless be identified on this medium by the characteristic translucent quality of the typhoid colony, which distinguishes it from the metallic sheen exhibited in most instances by even the smallest colony of the colon types. In case this distinctive appearance of the typhoid colony is not clearly perceived by daylight, this quality may be easily brought out by artificial illumination.

While this work was in progress, two plating mediums were reported; an eosin-methylene blue medium by Holt-Harris and Teague,[5] and an eosin-brilliant green medium by Teague and Clurman.[6] The eosin-methylene blue medium depends on the application of the protective colloid principle. The two dye-solutions which in the concentrations used would mutually precipitate in aqueous solution, are prevented from combining when added separately to the agar, due to the protective action of the colloids in the medium. When fermentation occurs in the development of a colony, the protective action of the colloids is destroyed by the products of fermentation at this point, precipitation occurs, and the fermenting colony assumes a contrasting color.

The eosin-methylene blue medium has no differential restraining action on B. coli, and was suggested as a substitute for Endo's medium. We have worked with this medium and agree with the observations of Holt-Harris and Teague as to the sharp differentiation of the fermenting and nonfermenting types, the absence of any diffusion even with heavy seeding, and the lack of sensitiveness on exposure of light. Since no diffusion occurs, a 1½ per cent. agar may be used as the basis to the medium instead of the 2½ per cent. agar usually required for Endo, which is a distinct advantage. Holt-Harris and Teague note one drawback to the eosin-methylene blue medium; the tendency of the reaction to disappear when growth is crowded, but claim that the medium will give successful differentiation, when an Endo plate equally inoculated would be unworkable on account of the diffusion of the indicator. We are not ready to agree with this contention. With a satisfactory Endo medium even an apparently hopeless plate can be fished by the aid of artificial illumination, the translucent, glassy typhoid colony being easily differentiated from the opaque, more deeply colored fermenting colonies which, even though small, nearly always show a distinctive metallic sheen. With diffusion on Endo, unless it is very intense, the search for the translucent typhoid colony seems to us no more difficult than on the equally deeply colored eosin-methylene blue agar. The colored background to our mind is the essential objection to the medium. In examining a large number of plates, it strains and tires the eyes rapidly, and obscures the differences between the typhoid colonies and other nonfermenting types, which are usually perceptible on Endo, to those accustomed to its use. Both mediums have advantages, and the selection must depend on personal preferences. For occasional or small series of examinations, the eosin-methylene blue medium would probably be preferable because of the stability of the reagents used and the resulting greater uniformity of the product. We have found the extract agar we use for the brilliant green plating medium, to be a satisfactory basis for the eosin-methylene blue medium. Because of the transparent background and the tendency of the typhoid colony to take on positive characteristics, a properly standardized Endo plate gives the best picture obtainable, and because of these facts as well as the large number of examinations, we have continued its use in spite of its other drawbacks.

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[5] Jour. Infect. Dis., 1916, 18, p. 596.
[6] Jour. Infect. Dis., 1916, 18, p. 647.

We have also tried the eosin-methylene blue medium with members of the paratyphoid and dysentery groups. With B. paratyphosus A and B, the results were as satisfactory as with B. typhosus. The dysentery types, particularly the Shiga, are apt to be irregularly restrained on this medium. Since these observations are based on stock cultures, they are only to be interpreted as a caution, as we have had no experience with freshly isolated fecal strains. We have noticed a similar tendency to irregular restraint, with dysentery strains on Robinson and Rettger's modification of Endo. Kendall's Endo has not shown this action if the plates were freshly poured.

The color change on the eosin-brilliant green medium of Teague and Clurman is less sharp than with the eosin-methylene blue medium. The essential claim made for the eosin-brilliant green medium was that the eosin acting probably by absorption reduces the activity of the brilliant green to a base level where it is no longer appreciably influenced by differences in the medium or by the fecal proteins inoculated. We have tried this medium to only a limited extent because of the difficulties encountered in obtaining what we considered a satisfactory end-product. These difficulties were in relation to obtaining suitable differential restraint and were probably due to the fact that a sufficient range of dilutions were not tried. In a later article, Teague and Clurman[7] suggest the preliminary use of several concentrations of brilliant green and that large batches of agar be prepared to limit the number of preliminary tests of the medium. In other words, the masking action of the eosin does not exclude the necessity of preliminary standardization and the determination of the optimum concentration of brilliant green in the routine examinations. There are other and more essential disadvantages to the medium. A meat-infusion agar is necessary. The comparatively deep color of the medium obscures any positive characteristics the typhoid colony may possess, eye-strain resulting from the attempt to differentiate the nonlactose fermenting types. The eye-strain is enhanced by the peculiarly irritating color of the medium when viewed by transmitted light. This becomes a very serious drawback when the plates to be examined by one individual run into the hundreds. Both these difficulties are further enhanced when the plates become crowded as the differential color reaction tends to disappear. Since standardization is necessary, an almost colorless transparent medium which, however crowded, still retains the characteristic appearance of the typhoid colony seems to us preferable.

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[7] Jour. Med. Research, 1916, 35, p. 121.

SUMMARY

The brilliant green medium we have devised has markedly increased the number of positive fecal examinations for B. typhosus and B. paratyphosus. The methods of preliminary and final identification previously recommended have been found reliable and have materially aided in making possible large numbers of examinations with the minimum of workers. The essential point in the success of the medium is proper standardization. No method has been devised to render such standardization unnecessary. The medium is best adapted to routine work where many specimens are examined, as in such case the time required for standardization is relatively slight, because large amounts of medium can be standardized and used before the results of the standardization tests are rendered void by the deterioration of the dye solution or evaporation of the medium.

Of the recently suggested nonrestraining plating mediums, Robinson and Rettger's modification of the Endo agar has given a far superior contrast between lactose and nonlactose fermentation types than that obtainable with the various modifications thus far tried. The methylene blue-eosin medium of Holt-Harris and Teague gives a similarly striking contrast if the plates are not crowded, but has the objection of being a colored medium thus obscuring any differences between the colonies of the nonlactose types and tiring the eyes when numerous plates are to be examined. Both of these mediums tend to restrain the growth of some culture strains of B. dysenteriae, indicating that they may not be satisfactory for the isolation of this organism from feces.

BRILLIANT GREEN AS A BACTERICIDAL AGENT FOR THE PURIFICATION OF VACCINE VIRUS.*

Charles Krumwiede, Jr., Frank S. Fielder, and Thomas A. Watson

As far as we know Churchman[1] is the only investigator who has made observations on the effect of strongly bactericidal anilin dyes on vaccine virus. He gives no details, stating simply that the potency of vaccine virus was unimpaired by staining with gentian violet.

We were led to investigate the influence of anilin dyes on the potency and bacterial content of vaccine virus because of the frequent presence of gas-producing anaerobic bacilli which give positive reactions with the official hygienic laboratory tests for the presence of B. welchii; and because of the continued viability of these bacilli in spite of the glycerole and carbolic acid added to purify and preserve the virus.

Brilliant green was chosen because of its wider range of bactericidal action[2] as compared with the violet dyes and especially because of its action on bacilli of the colon group.

The data presented in this article show that brilliant green has little, if any, influence on the potency of vaccine virus and when added to the present glycerol-carbolic preservative, gives a more efficient and more rapidly bactericidal preservative.

The accompanying table gives the bactericidal results obtained with virus artificially inoculated with the bacterial types considered most objectionable in virus.

Brilliant green added to heavily inoculated glycerol-carbolic virus led to a rapid reduction of the inoculated bacteria and sterility in six days, whereas with glycerol-carbolic alone the reduction was slower and sterilization was not complete in this length of time.

The following are examples:

Virus 2260, collected April 14, 1915; B. welchii types still present, March 1, 1916.

Virus 2261, collected April 28, 1915; B. welchii types found May 6, 1916, not found June 9, 1916.

Virus 2262, collected May 12, 1915; B. welchii types found May 16, not found June 9, 1916.

Virus 2263, collected May 12, 1915; B. welchii types found June 11, 1916.

In this connection it is well to note the persistence of the types of organisms in question when encountered in glycerol-carbolic virus as prepared ordinarily with 1 per cent. carbolic.

No quantitative estimations were made, but based on the amount of growth obtained in the primary "shake" cultures made from the virus, numerous spores were present at the end of the observation.

Tetanus spores are evidently highly resistant to brilliant green. This gives the dye an indirect advantage over the preservative in use at present in vaccine virus. If tetanus bacilli be present they should be recovered with ease due to the killing of the associated bacteria and spores. This applies especially to organisms of types related to B. welchii, as these by overgrowth may multiply the accepted tests for the presence of tetanus bacilli or spores.

Before instituting a series of tests of viruses with and without brilliant green, it seemed well to determine whether the carbolic acid was of added value when the dye was employed. It was found that with the carbolic

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* Published in Jour. Infect. Dis., 1918, 22, p. 118.

[1] Proc. Soc. Exper. Biol. and Med., 1914, 11, 55.

[2] Krumwiede, C., and Pratt, J. S.: Jour. Exper. Med., 1014, 19, 501.

TABLE 1.

VACCINE VIRUS TO WHICH WAS ADDED ONE-FOURTH OF ITS VOLUME OF A HEAVY MILKY SUSPENSION OF STAPHYLOCOCCI AND STREPTOCOCCI, KEPT IN REFRIGERATOR BETWEEN TESTS.

Dates Tested 0.1 c.c. Plated, Blood-agar.	Inoculated Glycerol-Carbolic Virus Containing Brilliant Green; Concentration, 1:10,000.	Inoculated Glycerol-Carbolic Virus; Saline to Same Volume as Preceding.
July 22, 1915....	Plates, solid very closely crowded fine colonies.	Plates as Preceding.
July 23.........	33% reduction in number of colonies.	No evident reduction.
July 24.........	40% reduction.	5% reduction.
July 26.........	99% reduction.	15% reduction.
July 27.........	Three colonies total on three plates.	50% reduction.
July 28.........	No growth.	98% reduction.

TABLE 2.

VIRUS CONTAINING LARGE NUMBERS OF BACILLI GIVING POSITIVE B. WELCHII TESTS WAS FURTHER INOCULATED WITH CULTURES OF A SIMILAR BACILLUS ISOLATED FROM PREVIOUS VIRUS. THE VIRUS WAS THE ROUTINE GLYCEROLE-CARBOLIC SUSPENSION.

Glucose-Fermentation Tubes Inoculated.	Inoculated Virus, Glycerol-Carbolic.	Inoculated Virus, Brilliant Green 1:5000.	Inoculated Virus, Brilliant Green 1:10,000
At time of mixing...	90% gas	No gas; probably dye inhibition	100% gas
After 2 days.......	95% gas	No gas	No gas
After 6 days.......	60% gas	No gas	No gas

TABLE 3.

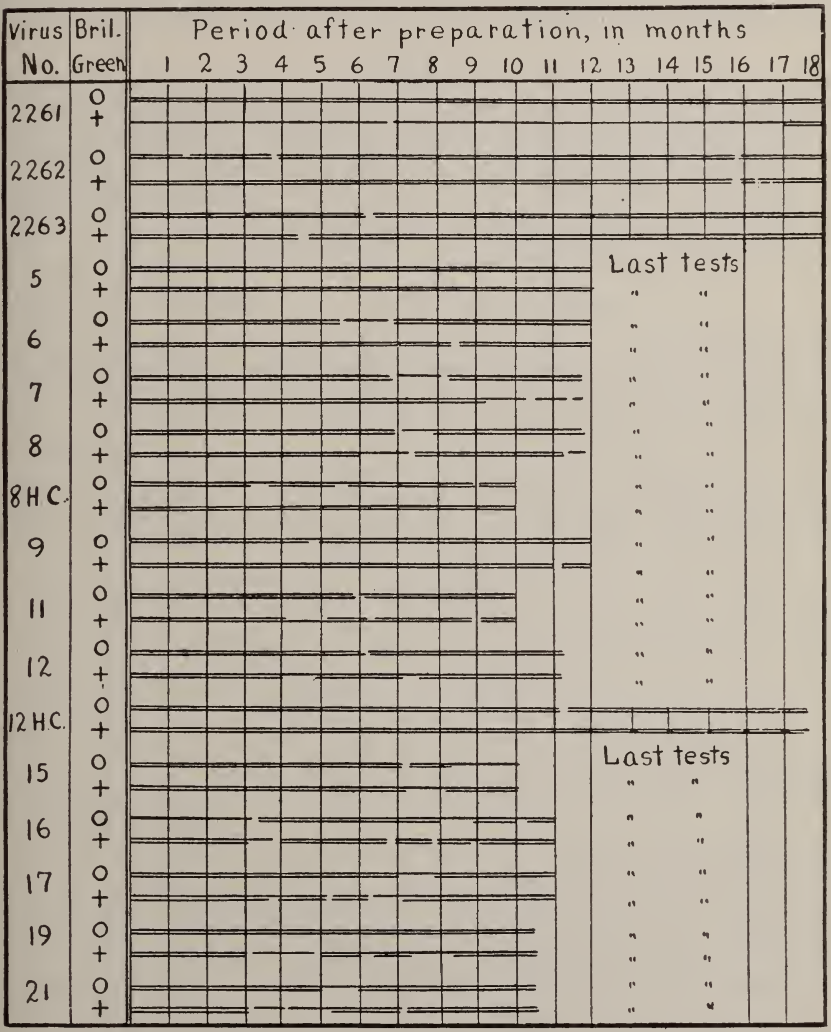
VIRUS HEAVILY INOCULATED WITH TETANUS SPORES OBTAINED FROM BROTH CULTURE SEDIMENT.

Tested.	Inoculated Virus, Glycerol-Carbolic.	Inoculated Virus, Brilliant Green 1:5000.	Inoculated Virus, Brilliant Green 1:10,000.
At once.........	Tetanus bacilli isolated.	Tetanus bacilli isolated.	Tetanus bacilli isolated.
After 248 days...	Tetanus bacilli isolated.	Tetanus bacilli isolated.	Tetanus bacilli isolated.

acid the disappearance of some undetermined types of cocci was more rapid. Comparative tests revealed that a concentration of at least 1 : 10,000 of the dye was necessary to obtain bacteriologic sterility in a relatively short time. A greater concentration, 1 : 5000, was not appreciably more effective, and as at this concentration there was some indication of injury to the vaccine virus itself, practically all the subsequent observations were made with a concentration of 1 : 10,000.

Evidently brilliant green when added to glycerol-carbolic virus markedly hastened its purification. As the first test on the brilliant green virus frequently gave negative cultures the earliest day on which a bacteriologically sterile preparation was actually obtained cannot be stated. Because of this,

Chart



Duration of potency of vaccine virus with and without brilliant green.

Double line=100% positive takes of highest quality. Single line=100% positive takes of moderate quality. Break in both lines=less than 100% takes. Break in one line=break in quality of takes. "Takes"=primary vaccinations of children. (We are indebted to Drs. Samuel Parnass and Julius Blum who carried out the clinical tests on children). += brilliant green added. O=no brilliant green, glycerol-carbolic virus.

the following comparison of averages is distinctly in favor not of the virus thus treated, but in favor of the glycerol-carbolic virus. Thus, with the brilliant green virus the average day on which gas was not noted was 11 days; growth not noted 24 days; with the glycerol-carbolic virus, gas was still positive after an average of 33 days and growth was still positive after an average of 119 days.

The accompanying chart gives a summary of the potency tests of vaccine virus with and without brilliant green. The concentration of brilliant green employed was 1 : 10,000.

Although variations in the degree of "takes" are sometimes in favor of the virus without brilliant green, the reverse also occurs. On the whole,

TABLE 4.

COMPARATIVE RATE OF PURIFICATION OF GLYCEROL-CARBOLIC VIRUS WITH AND WITHOUT BRILLIANT GREEN. TESTED BY INOCULATION OF GLUCOSE BROTH IN FERMENTATION TUBES. DAY NOTED OF LAST POSITIVE AND FIRST NEGATIVE TEST FOR GAS PRODUCTION; OF LAST DAY OF CLOUDING AND FIRST DAY OF ABSENCE OF GROWTH. TESTED ABOUT EVERY 7 TO 14 DAYS. GAS NOTED AS ITS DEVELOPMENT IS AN INDICATION OF THE PRESENCE OF B. WELCHII TYPES.

Virus Number.	Brilliant Green 1:10,000.				Brilliant Green not Added.			
	Gas.	Days.	Growth.	Days.	Gas.	Days.	Growth.	Days
14	+ —	2 3	+ —	11 20	+ —	30 39	+	92 ......
15	+ —	10 21	+ —	82* 91	+	93	+	206 ......
16	...... —	...... 9**	...... —	...... 9**	+	43	+	45 ......
17	...... —	...... 9**	...... —	...... 9**	+ —	4 17	+	45 ......
19	...... —	...... 16**	...... —	...... 16**	+	44	+	44 ......
21	+ —	1 8	+ —	24 29	+ —	32 42	+	199 ......
22	+ —	3 10	+ —	26 32	+ —	14 21	+	193 ......
23	...... —	...... 11**	...... —	...... 11**	+ —	13 31	+ —	156 171
24	...... —	...... 12**	...... —	...... 12**	+ —	4 13	+	147 ......
25	...... —	 9	...... —	 9	+ —	71 94	+ —	71 94
26	...... —	 13	...... —	 13	+ —	28 55	+	112 ......
27	...... —	 13	+ —	28 37	+ —	14 21	+ —	57 71

* Only organism after 2 months, small coccus.

** No previous tests.

there is no evidence of the dye exerting any appreciable deleterious influence, especially if the duration of potency is considered. Probably some of the variations in results are dependent on inherent variables in the method of testing.

As a control on the foregoing human vaccinations, vaccinations were carrier out on rabbits with dilutions of treated and untreated vaccine, as explained in Table 5.

The results recorded in Table 5 show no influence of the dye on the total content of virus after exposure for forty days.

We have employed brilliant green to preserve the seed-virus collected from calves vaccinated with human virus as well as to preserve the seed-virus from rabbits vaccinated with this human-calf virus. This rabbit virus is used to vaccinate calves in order to obtain virus for general distribution; by the interpolation of the rabbit, calf to calf inoculation, which commonly has a deleterious effect on the potency is avoided.

TABLE 5.

POTENCY TESTS ON RABBITS VACCINATED WITH VACCINE VIRUS WITH AND WITHOUT BRILLIANT GREEN DYE 1:10,000. VIRUSES 5 AND 6 WERE EXPOSED TO BRILLIANT GREEN, MARCH 31, 1916.

Virus Number and Date of Test.	Without Brilliant Green.	Results.	With Brilliant Green 1:10,000.	Results.
	Dilution of Virus.		Dilution of Virus.	
June 9, 1916.				
Virus 5	Undiluted	Good confluent take	Undiluted	Good confluent take
Virus 5	Diluted 1:10	Good confluent take	Diluted 1:10	Good confluent take
Virus 5	Diluted 1:100	Good confluent take	Diluted 1:100	Good confluent take
Virus 5	Diluted 1:1000	Good confluent take	Diluted 1:1000	Good confluent take
June 9, 1916.				
Virus 6	Undiluted	Good confluent take	Undiluted	Good confluent take
Virus 6	Diluted 1:10	Good confluent take	Diluted 1:10	Good confluent take
Virus 6	Diluted 1:100	Good confluent take	Diluted 1:100	Good confluent take
Virus 6	Diluted 1:1000	Good confluent take	Diluted 1:1000	Good confluent take

In Table 6 the seed exposed and the time of exposure are given.

The addition of brilliant green to the seed virus had no effect on the potency of the final virus. In no instance was any appreciable diminution noted in the intensity of the "take" nor in the amount of yield of virus from the animals vaccinated.

At first the vaccine virus was treated with brilliant green by adding 1 part of a 1 : 1000 solution of the dye to 9 parts of the finished glycerol-carbolic virus. Later the pulp from the calf was ground up in a glycerol-carbolic solution containing 10 per cent. less water which was replaced by a like volume of brilliant green solution (1 : 1000) ; thus any dilution of the finished product as compared with the routine glycerol-carbolic virus was avoided.

In observing the results of vaccinations made with the brilliant green-treated virus, there has been some indication that the secondary inflammation was reduced. Possibly this effect could be enhanced by subsequent applications of the dye-solution to the vaccinated area, limiting in this way the multiplication of the skin cocci.

The sterilization of the virus by brilliant green may find an important

TABLE 6.
TYPE OF SEED EXPOSED, TIME OF EXPOSURE AND RESULTS.

Human-Calf Virus; Number and Date of Exposure.	Rabbit Seed; Number and Date of Exposure.	Calf Vaccine; Number and Date Vaccinated.	Duration of Potency of Calf Vaccine in Days.
8 June 10, 1916	1 July 18, 1916	18 Aug. 9, 1916	Still good at 300 days
	2 July 25, 1916	19 Aug. 24, 1916	Still good at 294 days
		22 Sept. 29, 1916	Still good at 301days
12 June 10, 1916	1 Oct. 9, 1916	24 Nov. 17, 1916	Still good at 253 days
		25 Dec. 7, 1916	Still good at 233 days
	3 Nov. 8, 1916	27 Dec. 21, 1916	Still good at 218 days
		28 Jan. 23, 1917	Still good at 175 days
	4 Jan. 16, 1917	32 Feb. 8, 1917	Still good at 168 days
	5 Feb. 5, 1917	33 Feb. 21, 1917	Still good at 155 days

Explanation: Numbers refer to animal collections. From left to right gives passages of each collection from calf to rabbit to calf.

practical application in that intermediate sterilization would allow calf to calf vaccination, without loss in potency of the virus. Without this bacterial sterilization calf to calf inoculation leads commonly both to degeneration of the virus and decreasing yields; this is due apparently to a great extent to the overgrowth of the calf bacterial flora enriched by the transfer of this flora from calf to calf. At present this overgrowth is avoided by obtaining the seed for calf vaccination from rabbits.

The use of brilliant green alone, although somewhat less efficacious than in combination with carbolic acid, may be of service in obtaining a bacteriologically sterile virus for attempts at cultivation. The observation of Churchman and Russell[3] that certain animal tissues grow readily in concentrations of gentian violet which are bactericidal is suggestive in this connection.

CONCLUSIONS

Brilliant green in a concentration of 1 : 10,000 has no appreciable effect on the potency of vaccine virus. This amount of dye when used in combination with the glycerol-carbolic solution usually employed hastens markedly the rate of reduction of the bacterial content, rendering most preparations bacterially sterile in from two to four weeks.

Although the dye cannot be relied on to kill tetanus spores should they be present, no difficulty should be encountered in demonstrating their presence after the associated bacteria are destroyed.

The results obtained warrant the practical application of the dye to vaccine virus for general distribution or at least in emergencies when the virus must be employed shortly after collection from the calf.

The use of brilliant green gives a simple method hitherto not available for obtaining a bacteriologically sterile but fully potent virus for experimental purposes.

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[3] Proc. Soc. Exper. Biol. and Med., 1914, 11, 123.

A COMPARISON OF TRICRESOL AND CHLOROFORM AS A PRESERVATIVE IN ANTIMENINGITIS SERUM.*

Josephine B. Neal and Harry L. Abramson.

Until the fall of 1914, the Research Laboratory continued to use tricresol as the preservative in the antimeningitis serum, thus following the methods in use at the Rockefeller Institute. Late in 1914, the tricresol was replaced by chloroform on account of the discussion in regard to the toxic effects of tricresol, started mainly by reports of Kramer[1] of Cincinnati.

While the discussion on this subject is familiar to many, it may be summarized briefly: Kramer reported, in 1913, a series of cases in which death followed the administration intraspinally of serum containing tricresol as a preservative. He attributed these deaths to the tricresol, which he held depressed the medullary center, reaching it especially in young children through the central canal of the cord. Kramer also reported the results of experiments on dogs, which were not convincing. Flexner[2] next published an article in which he enumerated the various possibilities of danger in the use of the serum, namely: anaphylaxsis, liberation of toxin through the destruction of the meningococcus, and especially increased pressure. He pointed out that, since in the cases reported by Kramer the doses were large and were administered by a syringe, it was highly probable that increased pressure was the cause of death, and, furthermore, that accidents have ocucrred after the administration of serum containing no preservative. Hale[3] showed that the intraspinal injection of tricresol in dogs produced considerable respiratory difficulty which he interpreted as due to paralysis of the respiratory center. Fitzpatick, Atkinson and Zingher,[4] in our laboratory, showed that in dogs the pressure seemed to be the important factor, and that the gradual administration of serum with 0.3 per cent. tricresol did no harm. Auer[5] showed that this respiratory difficulty was much less marked in monkeys, and that it was not due to paralysis of the respiratory center, but to a stimulation of the inhibitory function.

One seems justified in concluding from Auer's experiments that while tricresol affects the medullary center of monkeys somewhat more than ether or chloroform, monkeys tolerate repeated large doses of serum containing tricresol, and that dangerous changes in respiration and blood pressure are due to increased intraspinal pressure. Auer's article contains a very comprehensive study of the subject from the experimental standpoint.

As stated above, serum containing tricresol, at first 0.3 per cent. and later 0.2 per cent., was used by the Meningitis Division of the Bureau of

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* Published in Jour. Am. Med. Ass., 1917, 68, p. 1035.

[1] Kramer, S. P.: A Possible Source of Danger in the Use of Antimeningitis Serum, The Journal A. M. A., May 3, 1913, p. 1348.

[2] Flexner, Simon: Accidents Following the Subdural Injection of the Antimeningitis Serum, The Journal A. M. A., June 21, 1913, p. 1937.

[3] Hale, Worth: The Cause of Death from Subdural Injections of Serum, Bull. 91, Hyg. Lab., U. S. P. H. S., December, 1913.

[4] Fitzpatrick, C. B.; Atkinson, J. P., and Zingher, Abraham: The Comparative Importance of Pressure and of Toxicity of Trikresol in Subdural Injections of Sera, Collected Studies from the Bureau of Laboratories, Department of Health, City of New York, 1914-1915, p. 37.

[5] Auer, John: The Effect of Intraspinal Injections of Serums, with and without Preservatives, The Journal A. M. A., June 6, 1914, p. 1799.

Laboratories from August, 1910, until December, 1914. During this time, the serum was administered about 600 times and to children of all ages, fourteen of the patients being less than 1 year old. As there had never been a fatality immediately following the injection, it seemed improbable to us that the tricresol could be a very dangerous factor even to young children, as Kramer had declared. In a few instances collapse had followed the administration of serum when large doses were used. Later, with smaller doses, these occurrences ceased.

In December, 1914, the use of the chloroform serum was begun. Those of the Division who had used both serums became convinced in time that the chloroform was the more irritating. This conclusion was not reached at once. There is, of course, a great difference in individuals in their reaction to pain. Moreover, the registration of pain in many of our patients is unreliable because of delirium, stupor, or infancy. In time, however, it was noted, in the case of a number of rational and intelligent patients who had behaved well during the puncture and withdrawal of fluid, that the injection of the serum was accompanied by excrutiating pain, so that they were unable to keep from crying out and struggling. This added greatly to their dread of the treatment, and to the objections of the family. The proper regulation of dosage was also interfered with, as the crying out and struggling increased the intraspinal pressure and prevented the easy introduction of the serum. Finally, one lot of serum containing tricresol was prepared and used so that comparative studies might be carried out in the same individual.

Case 1—A girl of 16 had received four injections of chloroform serum, suffering extremely each time, so much so that each injection had to be stopped before 20 cubic centimeters were given. She then received six injections of tricresol serum. These were accompanied by practically no discomfort. At the second injection she fell asleep before it was finished. It was also possible to increase the dose to 25-28 cc., which seemed desirable in this case as the infection was very resistant.

Case 2—A man, aged 19, had received several doses of chloroform serum accompanied by great pain each time. The tricresol serum caused practically no pain.

Case 3—A child of 4 years, who had received three doses of tricresol serum without resisting, struggled and cried during the administration of one dose of chloroform serum.

Case 4—An Italian boy of 5½ years complained of severe pain with the chloroform but not with the tricresol.

Case 5—A baby was given one dose of tricresol serum without any marked reaction. When given a dose of chloroform serum, the baby struggled violently.

Case 6—A boy of 12 years struggled furiously, three men being needed to restrain him, during two injections of chloroform serum. He was quiet during the administration of tricresol serum.

These six cases in which both tricresol and chloroform serum were used, in addition to our observations in cases in which either the chloroform or the tricresol serum alone was received, show very conclusively that chloroform serum is far more painful than tricresol serum. It may be stated that the injection of serum is practically always *followed* by pain in the legs and general discomfort. It is the sensation *during* injection to which we refer.

The observations in the cases reported above were made by several other physicians as well as by members of the Meningitis Division.

It is possible, since it belongs to the phenol group, that tricresol may, like phenol (carbolic acid) exert a local anesthetic action. Indeed, it would seem that it must be so, as chloroform is added to practically all the serum used when it is first withdrawn from the animal; consequently the tricresol serum contains tricresol in addition to the chloroform.

Whether or not because of the difference in the preservative of the serum, it may be stated that the mortality of cases treated by the Meningitis Division from Dec. 1, 1913, to Dec. 1, 1914, the period during which all the serum contained tricresol in addition to the chloroform, was 30 per cent., while for the following year, when chloroform was present without tricresol it was 38 per cent.

Tricresol has a marked bactericidal action, in vitro, on the meningococcus, and also exerts within forty-eight hours some bactericidal effect on organisms that might accidentally contaminate the serum. Chloroform possesses much less bactericidal action and would, therefore, seem to be of limited value as a preservative.

The above points are illustrated in the following experiments:

Experiment 1—Bactericidal Action of Chloroform in 0.2 Per Cent. Dilution—Before the experiment was begun, normal horse serum was freshly drawn and tested for sterility. Twenty-four hour cultures on glucose ascitic agar showed no growth. Direct dilution of the chloroform was made in 50 per cent. of this serum to secure a better solubility of the chloroform.

The organisms used were: the colon bacillus, the staphylococcus, the streptococcus and the meningococcus (second generation) after each had been grown on glucose ascitic agar for 24 hours. Three c.c. of sterile saline solution were used in washing off the growths of each organism except the streptococcus, for which two cubic centimeters of saline solution were used in order to obtain a heavier suspension of bacteria. These washings constituted the bacterial suspensions for the experiment.

After the bacterial suspension and the chloroform dilutions had each been thoroughly shaken, equal quantities of the two materials were mixed and shaken up for a few minutes. The bacteria were then left exposed to the action of the disinfectant for periods of time of half an hour, one hour, two hours, four hours and twenty-four hours. At the expiration of each period of exposure two loopfuls of the mixture were planted on slants of glucose ascitic agar, and all the slants were placed in the incubator at 37.5 C.

Control cultures were made of the serum dilutions, bacterial suspensions, and the saline solution used throughout the experiment.

TABLE I.

EXPERIMENT I.

Organisms and Controls.	Exposure Time.				
	½ Hour.	1 Hour.	2 Hours.	4 Hours.	24 Hours.
B. coli	++++	++++	++++	++++	++++
Staphylococcus	++++	++++	++++	++++	++++
Streptococcus	++++	++++	++++	++++	++++
Meningococcus	++++	++++	++++	++++	++++
Serum control	—	—	—	—	....
Bacterial suspension control	+	+	+	+	....
Saline solution control	—	—	—	—	....

Experiment 2—Bactericidal Action of Tricresol in 0.1, 0.2 and 0.3 Per Cent. Dilutions on the Meningococcus in 100 Per Cent. Serum.—In this experiment, the procedure was the same as that for Experiment 1. The disinfectant dilutions were made up directly in normal serum, a 100 per cent. serum being used. A strain of meningococcus of the second generation was used.

TABLE 2.
EXPERIMENT II.

Dilution, Per Cent.	Exposure Time.				
	½ Hour.	1 Hour.	2 Hours.	4 Hours.	24 Hours.
0.1	++++	++++	++++	++++	—
0.2	++++	++	—	—	—
0.3	—	—	—	—	—

Experiment 3—Bactericidal action of Tricresol in 0.1, 0.2 and 0.3 Per Cent. Dilution on Such Organisms as Might Commonly Contaminate a Serum, in 50 Per Cent. Serum.—This experiment was carried out according to the same method used in Experiment 1 and Experiment 2.

TABLE 3.
EXPERIMENT III.

Organisms and Controls.	Exposure Time.					
	½ Hour.	1 Hour.	2 Hours.	4 Hours.	24 Hours.	48 Hours.
B. coli.						
0.1 per cent. dilution	++++	++++	++++	++++	++++	++++
0.2 per cent. dilution	++++	++++	++++	+++	+++	+++
0.3 per cent. dilution	....	....	....	....	—	—
Staphylococcus.						
0.1 per cent. dilution	++++	++++	++++	++++	++++	++++
0.2 per cent. dilution	++++	++++	++++	++++	++++	++++
0.3 per cent. dilution	++++	++++	++++	++++	++++	++++
Streptococcus.						
0.1 per cent. dilution	++++	++++	++++	++++	++++	++++
0.2 per cent. dilution	++++	++++	++++	++++	+++	+
0.3 per cent. dilution	++++	++++	++++	++++	—	—
Bacterial suspension control	+	....	....	....	+	+
Serum control	—	....	....	....	—	—
Saline solution control	—	....	....	....	—	—

CONCLUSIONS.

1. The bactericidal action of tricresol on possible contaminating bacteria in serum is superior by far to that of chloroform.

2. The marked bactericidal action of the tricresol shown on the meningococcus in vitro may be of advantage in vivo.

3. When chloroform, not tricresol, is used as a preservative in serum the pain of injecting the serum is so great as to constitute a serious drawback in the treatment of meningitis, not only on account of the resulting objection on the part of the patient and the family, but also on account of the difficulty of regulating the dosage when the patient is struggling.

THE RELATION OF THE INTESTINAL FLORA TO THE SCURVY OF GUINEA PIGS AND OF INFANTS.*

J. C. Torrey and Alfred F. Hess.

The question has been raised recently as to whether scurvy in guinea pigs and in human beings is due to constipation and to the putrefactive activity of the bacteria in the intestinal tract. In order to determine this point a study of the intestinal flora was made in guinea pigs on a normal diet, on a diet which produced scurvy, and again on a diet which cured this disorder. For this purpose cultures and Gram stained films were made from the feces and from the contents of the large and small intestines at different levels immediately after chloroforming the animals. This study on guinea pigs is portrayed in Table 1.

It will be seen that proteolytic bacteria other than subtloid types were not found in the intestinal tract; that the bacteria which appeared in the cultures were merely those found in the outer world, for example, on dried foodstuffs. Similar organisms, indeed, were cultivated from the hay and oats which were fed these animals. Attention may be called to the fact that with none of the diets did B. coli develop, and that it was isolated from only one animal. The number of viable bacteria will also be noted as remarkably few, generally less than 1,000 per milligram of material. Among these there were hardly any that were actively proteolytic. Furthermore, a point of prime importance, there was no change in the flora on adding orange juice to the diet, although the scorbutic symptoms disappeared. It is our opinion, therefore, that most of the bacteria entering the intestinal tract are destroyed and that the pabulum is not such as to encourage the growth of native intestinal bacteria.

A similar study was carried out on the stools of three infants suffering from scurvy. Table II gives a summary of these results. It will be seen that the infants were all on a high carbohydrate diet, and that in two instances we were able to compare the flora, not only during the active scorbutic process, but after orange juice had been given for a week or more. This investigation shows that the bacteria found were merely such as we should expect with a diet rich in carbohydrates. Putrefactive organisms were present only in small numbers; in the cases where they were found to be most numerous they had disappeared upon the subsequent examination, although the scurvy had become more marked.

We therefore conclude from this study that the scurvy, both of guinea pigs and of infants, is not associated with an overgrowth of putrefactive bacteria in the intestinal tract. The amount of putrefaction present, however, can be determined only by a chemical study.

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* Published in Proc. Soc. Exp. Biol. and Med., 1918, 15, p. 74.

TABLE I.

INTESTINAL FLORA OF NORMAL AND OF SCORBUTIC GUINEA PIGS.

Guinea Pig.	Diet.	Period of Feeding.	Scorbutic Condition.	Source of Material Cultured.	Types of Bacteria.	Remarks.
No. 1664	Straw, oatmeal, water			Cecum	Staph. alb. and aur. Subtiloid Gram neg. bact. Yeasts.	No B. coli. Very few viable bacteria.
		20 days	Scurvy	Colon	Subtiloid Gram+diploc. B. acidoph. Yeasts	No B. coli. Material almost sterile.
				Ileum	Staphyloc. Gram+diploc. Yeasts	No B. coli. No putrefactive types. About 1 viable organism per mg.
H. 63	Straw, oats, water	19 days	Scurvy	Cecum	Subtiloid bact. Gram+cocci B. acidoph. Yeasts	About 1 viable organism per mg.
				Colon	Like cecum	Like cecum.
No. 414	*Carrots*, hay, water	Several weeks	Normal	Feces	Subtiloid bac. B. acidoph. Gram+diploc. Yeasts	No B. coli (or other gas-producing bact.) 20,000 viable organisms per mg.
	Oats, hay, water	8 days	Normal	do.	Flora unchanged	No B. coli. 1,000 viable organisms per mg.
	do.	15 days	Scurvy	do.	Gram neg. bact. (saprophytic) Staph. alb.	No B. coli. 16,000 viable organisms per mg.

TABLE I.—*Continued.*

Guinea Pig.	Diet.	Period of Feeding.	Scorbutic Condition.	Source of Material Cultured.	Types of Bacteria.	Remarks.
	Oats, hay, *orange juice*, water	3 days	Normal	do.	Subtiloid bac. (spore-bearing) Gram+diploc. Yeasts	No B. coli. 56 viable organisms per mg.
	Do.	8 days	Normal	Ileum	Streptoc. Gram+cocci B. acidoph. Yeasts	No B. coli. 1,700 viable organisms per mg.
				Cecum	Moulds Yeasts Spore-bearing bac. (numerous) M. ovalis	No B. coli. 640 viable organisms per mg. Putrefactive tendency.
				Rectum	Yeasts Streptoc. B. acidoph.	No. B coli. 840 viable organisms per mg.
No. 1776	Raw goat's milk, hay	87 days	Scurvy	Ileum	Yeasts B. acidoph. Saprophytic bact.	No B. coli. No proteolytic bact. 264 viable organisms.
				Cecum	Yeasts B. acidoph. Gram neg. twisted bact. M. ovalis.	No B. coli. No proteolytic bact. 560 viable organisms.
				Rectum	Yeasts B. acidoph. Saprophytic bact.	No B. coli. No proteolytic bact. 4,380 viable organisms.

TABLE II.

FECAL FLORA OF SCORBUTIC INFANTS.

Infant.	Diet.	Date.	Scorbutic Condition.	Source of Material.	Types of Bacteria.	Remarks.
M.	Malt soup and cereal	Dec. 1	Subacute	Rectum	B. acidoph. B. bifidus M. ovalis B. coli	Normal infants' flora. Gram+bact. predominant. No spore-bearing or putrefactive types. B. acidoph. about 40% viable bact.
	Do.	Dec. 4	Do.	Do.	Do.	Do.
	Same also, 20 c.c. liquid petrolatum, one week	Dec. 11	Subperiosteal hemorrhage	Do.	Do.	Relatively more B. coli. Many B. bifidus. No putrefactive bact.
	Same diet, oil stopped, *orange juice*, 10 days	Dec. 21	Markedly improved	Do.	Do.	Normal infants' flora. Bacteria as above.
K.	Malt soup and cereal	Dec. 11	Subperiosteal hemorrhage	Rectum	Streptococci B. coli M. ovalis	Gram neg. bacteria predominant. B. coli gram+diploc. numerous. B. acidoph. few. B. bifidus few.
	Do.+*orange juice* (60 c.c.), 8 days	Dec. 21	Markedly improved	Do.	B. bifidus B. coli Streptococci	Gram+bact. predominant. Many B. bifidus. Streptoc. unchanged.
S.	Formula: Cream, water, flour, sugar, also cereal	Dec. 21	Mild scurvy	Rectum	B. lact. aerog. B. coli M. ovalis B. bifidus B. welchii	Gram+and neg. bact. about equal. Many lact. aerog. Putrefactive bact. in minority.
	Malt soup and cereal	Feb. 11	More marked	Do.	B. lact. aerog. M. ovalis B. bifidus Dipheroids	Gram + bact. in great majority (B. bifidus). Spore bearers very few. Flora not at all putrefactive.

A MODIFICATION OF THE HYGIENIC LABORATORY METHOD FOR THE PRODUCTION OF TETANUS TOXIN.*

HARRIET LESLIE WILCOX

In the November, 1915, issue of the Journal of Medical Research,[1] Anderson and Leake briefly describe the method used at the Hygienic Laboratory, Washington, D. C., for the production of a uniformly potent toxin. The method in use at the Research Laboratory is essentially that given by Anderson and Leake with a few slight variations. As there have been many inquiries as to how we obtain our potent toxin it was thought that the details of the exact procedure might be of sufficient value to publish.

STOCK CULTURES

The stock cultures are grown on a semi-solid medium made as follows:

Veal broth	1000 c.c
Agar	5 grams
Witte's Peptone	10 grams
NaCl	5 grams
Reaction	Neutral to phenolphthalein

This medium is put into tubes about eight to ten cubic centimeters to a tube. The tubes are then autoclaved at fifteen pounds pressure for one-half hour, when they are ready for use.

To transfer stock cultures one tube of the semi-solid agar stock cultures is melted and this melted culture is used to inoculate tubes of the above semi-solid agar, the medium in these tubes having been freshly melted to expel the air, and then cooled to 50° C. One cubic centimeter of the culture is added to each tube, at least ten sub-cultures being made in this way from one tube of stock culture. After inoculation the tubes are cooled promptly, the plugs immersed in paraffine and the tubes incubated at 37° C. After one week's incubation the cultures are stored in the ice chest, where they may be kept for six months without affecting their ability to produce toxin.

PRELIMINARY CULTIVATION

Fill potato tubes with about 40 cc. of the glucose broth formula given below and sterilize in the Arnold for one and one-half hours on the first day and one hour on the second day. To make the first transfer for the preliminary cultivation, heat two tubes of glucose broth in Arnold for fifteen to twenty minutes to expel the air and then cool down to about 50° C. Add 8 cc. of the melted contents of a tube of semi-solid agar stock culture of B. tetani to each of the above tubes. These tubes are incubated for twenty-four hours and the next day, two freshly heated tubes of glucose broth are inoculated with 5 cc. of the glucose broth cultures planted on the previous day. On the third day, determine the number of flasks that are to be inoculated and inoculate as many freshly heated glucose broth tubes from the second glucose broth generation as there are flasks. Anderson calls for at least six or seven transplants in the glucose broth before the inoculation of the toxin broth, but at the Research Laboratory it has been found that after three generations or even two if need be, a toxicity of 1-25,000 or higher has been produced in the toxin broth.

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* Published in Jour. Bact., 1916, 1, No. 3.

[1] Anderson and Leake, 1915. A Method of producing Tetanus Toxin, Journal of Medical Research, 33, 239.

The Broth for Preliminary Cultures and Toxin Production

The following broth is used both for the toxin and for the preliminary cultures for inoculating the toxin broth:

To every pound of market veal, add 1000 cc. of water and place in ice chest over night. The next morning the infusion is placed over a free flame and raised to 45° C. and held at that temperature for one hour; it is then boiled briskly for one-half hour and the broth strained through cheesecloth. The amount of filtrate is measured and the following added:

Witte's Peptone	1.0 per cent.
Glucose (anhydrous)	1.0 per cent.
NaCl	0.5 per cent.

When the above ingredients are added, the broth is boiled until the peptone, glucose, etc., are melted, and is then titrated with $\frac{N}{10}$ NaOH. After the reaction has been corrected to +1 to phenolphthalein, room temperature titration, the flame is turned out. The broth is then filtered through two layers of absorbent cotton directly into 2 litre Erlenmeyer flasks, leaving only sufficient space in the flasks for the expansion of the broth during the sterilization in the Arnold. The flasks are sterilized for one and one-half hours on the first day and one hour on the second day.

Inoculation of Toxin Broth

After the second sterilization in the Arnold, the flasks are ready for inoculation. The broth may be cooled down to 55°-60° C. by allowing the flasks to stand at room temperature, or in a more rapid way by placing the hot flasks in a large sink, to which cool, and then cold, water is added until the lower portions of the flasks are covered. When the bottoms of the flasks are cool to the hand, the portions above the water being still very hot, the inoculation may be made as follows:

The plugs are carefully removed, the necks flamed and the plugs replaced. In a similar way, the mouths of the culture tubes are sterilized and then, partly removing the plug of a flask, the contents of a potato tube is poured rapidly into the flask. If one prefers, the broth culture may be transferred by using a pipette, but the former method has been used here without subsequent contamination and has been found very satisfactory. After inoculation, the flasks are incubated for fifteen days at 36°-37°C., care being taken to exclude all light from them.

The flasks at the end of twenty-four to forty-eight hours' incubation show a diffuse cloudiness with the formation of gas bubbles on the surface of the broth. Toward the end of two weeks, the gas bubbles usually disappear, while the cloudiness persists and a light precipitate forms at the bottoms of the flasks. If it is not convenient to filter on the fifteenth day, the cultures may be kept in the incubator until the twentieth day without a loss of toxicity but from the twentieth day to the twenty-fifth day, the toxin loses about 20 per cent. in potency.

Filtration

All glassware, fiters, etc., should be neutral to phenolphthalein and the greatest care should be taken to exclude both direct and indirect light, by darkening the room and by covering the filtering apparatus with dark cloths, ordinary black cambric being used at the Research Laboratory.

The broth cultures are first passed through Buchner filters about eight inches in diameter, which have been packed with a layer of finely shredded paper pulp 0.25 inch in thickness. It is of importance that the pulp should be so well packed that the filtrate is absolutely clear, otherwise it will clog the Berkefeld filter. The first filtrate, about 200 cc., which passes through the Buchner is discarded, as it contains a considerable amount of water from the pulp, after which the filtering of the toxin may be carried on. If, after passing eight to fourteen litres through the pulp, the filtrate begins to appear cloudy, the pulp must be discarded and the Buchner repacked. The clear filtrate is then passed through a sterile Berkefeld filter, and ten per cent. of a five per cent. solution of carbolic acid solution is added to the toxin which is now placed in the ice-chest, ready for testing its potency.

POTENCY TEST

Two 350 gram guinea pigs are inoculated subcutaneously on the abdomen with 1 cc. of a dilution of $\frac{1}{15,000}$ and $\frac{1}{25,000}$ of the toxin respectively. If the toxin has a potency of $\frac{1}{25,000}$ the pig receiving the $\frac{1}{15,000}$ dilution will die on the second to third day and the pig receiving the $\frac{1}{25,000}$ dilution should die on the fourth day. If both pigs die with symptoms of tetanus before the fourth day, the toxin is stronger than $\frac{1}{25,000}$ and a higher dilution should be tested.

No comparative tests as to stability have been made with toxin produced under the usual anaerobic conditions and by the Hygienic Laboratory method. The toxin produced by the Hygienic Laboratory method showed a somewhat unexpected stability, however, when tested in one instance, as follows:

	Filtered	Tested	Toxicity
Lot 25	7–24–14	7–24–14	1–25,000
		8–31–14	1–25,000
		9–30–14	1–20,000
		10– 4–14	1–20,000

The test was made on a small amount of toxin, about 30 cc., kept in an ordinary test tube in the ice-box and protected from the air only by a shallow covering of albolene.

DISCUSSION

From the above, it will be seen that our method for growing the tetanus bacillus for toxin varies very slightly from that of the Hygienic Laboratory. Instead of beef, we use market veal which has given almost invariably a toxin of $\frac{1}{25,000}$, occasionally even going as high as $\frac{1}{40,000}$ or higher. It is interesting to note that with the bob veal, that is, veal under the legal age limit for selling, we have rarely obtained a tetanus toxin above $\frac{1}{15,000}$ whereas the market or older veal used as control has produced a toxin of $\frac{1}{25,000}$ strength. This is in marked contrast to our results in the production of diphtheria toxin, since the bob veal has always given more potent diphtheria toxin than the older veal.

Anderson recommends a longer period of preliminary cultivation, and advises that tetanus cultures should be carried on from one to three weeks, by daily transfers. On the other hand, with only three transfers in glucose broth before inoculating the toxin flasks, we have obtained a toxicity of

1-40,000. Furthermore, by inoculating the glucose broth directly with a melted semi-solid agar culture of B. tetani, we have secured a toxin of over $\frac{1}{25,000}$ in strength, the control test which had been inoculated from preliminary broth cultures giving the same degree of toxicity. Confirmatory tests are being made along this line.

Here it might be well to state that we obtained different results from those recorded by other writers in regard to the growth in the preliminary cultures. Anderson and Leake found that a good growth is obtained in the first generation in 48 hours, in 24 hours in the second and third transplants, and in 16 hours in the fourth. At the Research Laboratory, we have found an abundant growth in eighteen to twenty-four hours in the first generation in glucose broth from the semi-solid; this profuse growth continues in the subsequent cultures up to the third or fourth transfer when there is a diminution, until in the sixth or seventh, it frequently happens that there is no growth or very slight growth after forty-eight hours or more. That is, out of thirteen cultures transferred from the sixth transfer in glucose broth there may be only six or seven tubes which show signs of growth, even after several days incubation.

At the Hygienic Laboratory, glucose agar stab cultures are used for growing the stock strains, but it is not stated whether these cultures are grown anaerobically or aerobically. The semi-solid agar that we are using for the stock cultures is especially satisfactory as the medium previously heated to expel air provides the only anaerobic conditions necessary to produce a heavy growth of B. tetani with spore formation after a few days incubation.

It is in the hope that other laboratory workers may find the above technique of practical aid in producing a uniformly potent toxin that these minute details have been given.

A MODIFIED INTRADERMAL TEST IN RABBITS FOR DETERMINING THE VIRULENCE OF DIPHTHERIA BACILLI.*

Abraham Zingher.

A simple and economical method for testing the virulence of diphtheria bacilli on guinea-pigs was presented in a previous paper. By this method two large guinea-pigs are used for determining the virulence of four different strains of diphtheria bacilli. Fresh twenty-four hour Loeffler cultures of the various strains to be tested are suspended uniformly, each in 20 to 25 c. c. of salt solution; and 0.2 c. c. of each suspension is injected *intradermally* into the depilated abdominal skin of a guinea-pig. The abdominal surface is divided for this purpose into four areas and the injections are made as far apart as possible. One animal serves as a control and receives intraperitoneally at the same time as the test injection 1.0 c. c. of antitoxin (about 500 units). The results of the tests are noted every twenty-four hours for four to five days. Virulent strains produce a definitely circumscribed local inflammatory lesion, which shows a superficial necrosis in two or three days. In the control pig, the skin at the site of the test remains normal. With non-virulent strains, no lesion will be found in either control or test animal.

Recently, while carrying on some work with toxin-antitoxin in rabbits, I used the intradermal test with diphtheria toxin to determine the development of an active antitoxic immunity to diphtheria in these animals. The accurate results obtained and the sharply circumscribed skin lesions produced by the toxin when no antitoxin was present suggested the routine use of rabbits instead of guinea-pigs for determining the virulence of diphtheria bacilli. For this purpose the back and flanks of the rabbits are shaved, or the animals are anesthetized and the hair is pulled out. Six intradermal injections can be easily made on one animal. Similar injections are made in a control animal that has received 500 to 800 units of antitoxin intravenously. The bacterial suspension is prepared in exactly the same way as for the test in guinea-pigs. If the strain is virulent a local indurated and necrosing lesion will develop at the site of injection, while the control test will show no lesion.

If the strain is non-virulent no induration and necrosis will develop. Occasionally with non-virulent strains a temporary redness and slight infiltration may appear at the site of injection in twenty-four hours. This will disappear, however, in another twenty-four hours and will not be mistaken for the lesion given by a virulent strain. Repeated comparative tests made on guinea-pigs and rabbits with numerous virulent and non-virulent strains have shown an absolute analogy in the results.

The advantages of using rabbits in preference to large guinea-pigs are many. Six strains can be easily tested on each rabbit instead of four on the guinea-pig; again, since rabbits are a little more resistant to diphtheria toxin than guinea-pigs, a somewhat larger number of bacteria can be injected in each area without the danger of killing the animal; further, the areas of injection can be a little more separated, thus avoiding a possible overlapping of lesions. At times, also, guinea-pigs are quite difficult to obtain and rabbits may have to be used for these tests.

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* Published in Proc. N. Y. Path. Soc., 1917, 11, 1-5.

RESEARCH ON THE DETECTION OF ADDED WATER IN MILK.*

HALSEY DURAND AND RESTON STEVENSON.

At the annual meeting of the American Chemical Society, held in New York City, September 27, 1916, one of the authors read a paper on "The Detection of Added Water in Milk."[1] In this paper the importance of devising a rapid and accurate method was brought out and a review of the existing methods was presented.

The *first* method discussed was the determination of the refractive index of the milk serum, which, while having the advantage of speed, has proved unreliable for the detection of less than 10 per cent. of added water, vastly too great a margin. The *second* method depends upon the determination of the freezing point,[2] the elevation above that of normal milk indicating the percentage of added water; this is an excellent method, but one requiring too much time for a routine laboratory. A *third* method, the determination of the specific gravity of the whole milk and of the serum, was also mentioned as having been in use for many years, but never having given satisfactory results.

The above paper contained a brief outline of proposed methods for further research. The results of these studies are the subject of the present contribution.

I—The first of these methods to be investigated was based on the theory that added water would increase the solubility of inorganic salts in the serum. A series of determinations was made using a weighed amount of anhydrous lead subacetate. The reagent was that used in Horne's methods for sugar analysis. Experiment showed that 3 grams were required for the coagulation of 100 cc. of milk. After the addition of the subacetate, the contents of the flask were vigorously shaken to coagulate the milk thoroughly. The contents of the flask were poured on a dry folded filter and 50 cc. of the serum collected for the determination. The lead was precipitated with 25 per cent. of sulfuric acid, and alcohol added to facilitate the precipitation. The lead sulfate was collected in an alundum crucible, washed with water and finally with alcohol, ignited and weighed. Several determinations were made by the above method.

The results proved unreliable, in that a variable amount of the subacetate in solution is adsorbed by the coagulum, and that, owing to the slowness of the filtration, it proved impractical to wash out all of the organic matter not coagulated, this latter causing a reduction of a portion of the lead sulfate when ignited.

II—A second series of determinations was made, in which weighed amounts of finely divided silver nitrate were used for the coagulation. The procedure was the same as described under I. The silver in 50 cc. of the serum was determined by titration with $N/10$ ammonium sulfocyanate, after addition of nitric acid and using ferric ammonium sulfate as indicator. This method is much more accurate and rapid than Method I, but the results show that the differences in the number of cc. used, in milks of

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* Published in Jour. Industrial and Engineering Chem., 1918, 10, p. 26.

1. This Journal, 9 (1917), 44.

2. Chem. News, 110, No. 2870, p. 259; No. 2871, p. 275; No. 2872, p. 283.

TABLE I.

PRELIMINARY EXPERIMENTS.

Cell No.	Sample.	No.	Temp. C.	Conductivity.
1	Whole Milk *a*...........	1	25.2	0.00527
	b...........	2	25.17	0.00520
	c...........	3	25.15	0.00520
	b + 10% Distilled Water.	4	25.10	0.00479
	a + 10% Croton Water..	5	25.17	0.00478
	c + 10% Distilled Water.	6	25.07	0.00466
	Whole Milk *a*...........	7	24.95	0.00513
2	Milk from individual cow.	1	25.0	0.00522
		2	25.0	0.00523
		3	25.0	0.00522
	Suspicious sample.......	4	25.0	0.00549
		5	25.0	0.00517
	Sealect, Grade A........	6	25.0	0.00503
	After standing in ice-box for 2 days...........	7	25.0	0.00585
		8	25.0	0.00530
		9	25.0	0.00521
		10	25.0	0.00571
		11	25.0	0.00624
		12	25.0	0.00755
		13	25.0	0.00560
		14	25.0	0.00515
		15	25.0	0.00569

TABLE II.

TESTS USING TEST TUBES AS CELLS.

Temperature 25° C.

Sample.	No.	Conductivity.
Authentic sample..	1	0.00543
Authentic sample..	2	0.00504
Same as 2........	3	0.00504
Same as 1........	4	0.00518
Same as 2........	5	0.00475
Authentic sample..	6	0.00504
Authentic sample..	7	0.00495
Authentic sample..	8	0.00460
Authentic sample..	9	0.00563
Same as 1........	10	0.00559
Same as 2........	11	0.00605
Same as 3........	12	0.00712
Same as 4........	13	0.00727

TABLE III.

TESTS USING CELL No. 2 ACCURATELY FILLED TO MARK.

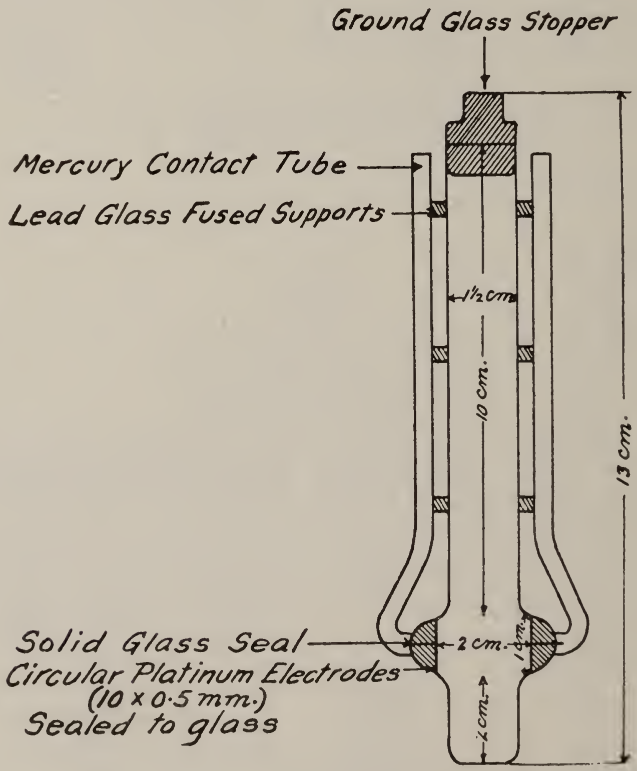
Temperature, 25° C.

Sample.	No.	Conductivity.
Borden's Grade B, pasteurized........	1	0.00519
Borden's Grade B, pasteurized........	2	0.00519
Locust Farm Grade B, pasteurized.....	3	0.00516
Milk from individual Holstein cow (Morning milking, unpasteurized)...	4	0.00519
	5	0.00516
	6	0.00519
	7	0.00513
	8	0.00519
No. 4 + 10% distilled water.........	9	0.00478
+ 10% distilled water.........	10	0.00478
+ 20% distilled water.........	11	0.00430
+ 50% distilled water.........	12	0.00307
+ 1.3% distilled water.........	13	0.00513
+ 3% distilled water.........	14	0.00503
+ 5% distilled water.........	15	0.00491
+ 7% distilled water.........	16	0.00482
+ 10% distilled water.........	17	0.00477
Grade A milk from ½ bottle (2 days old)	18	0.00503
Cream from No. 8..................	19	0.00439
Milk from 1 after removal of cream....	20	0.00532

known purity, are sometimes greater than those between whole milk and samples watered in the laboratory. The method was, therefore, abandoned as useless.

III—The third method,* which has been tried out by the authors in an exhaustive manner during the past year, consists in the determination, by the method of Kohlrausch, of the electrical conductivity of the whole milk and of the serum after coagulation with electrolytes and non-electrolytes.

The first series of experiments (Table I) was performed in a cell used for conductivity experiments in the laboratory, in which the electrodes were kept at a uniform distance, but were placed in the cell after filling without regard to their proximity to the sides. This cell was designated as No. 1. A constant temperature was maintained during all the following determinations. This was accomplished by warming the water with a coil of resistance wire (the current in the coil being regulated by a rheostat).



Stirring was done by bubbling compressed air through the mixture. It will be observed from Table I, Cell 1, that there is a decided similarity in the results on whole milk, and also that the milks to which water has been added give a lower conductivity. This follows the theory proposed.

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* Through the courtesy of Dr. Charles Baskerville, director of the Department of Chemistry, of the College of the City of New York, permission was obtained to carry on these experiments in the laboratories of that institution.

In the second series of experiments, Table I, Cell 2 was used, a cell similar in construction to Cell 1 but of greater capacity and with larger electrodes than the above.

Here as with Cell 1, the samples of known purity gave excellent results. The results on the samples marked, "in ice box 2 days," are not to be relied on, as change in composition has probably taken place.

In the experiments given in Table II, test-tubes, of the same commercial size, were used to facilitate the work, as a number of tubes could be placed in the bath at the same time, to bring them to the required temperature. The same methods were used as in Table I. In this series a wide deviation was observed on different tubes containing the same milk, amounting at most to 37.0 per cent. in the conductance. This was found to be due to the varying amounts of the sample contained in the tubes owing to their difference in size. To avoid a continuance of these errors a file mark was made on Cell 2 and in the subsequent determinations the cell was carefully filled to this mark.

The results in Table III for the whole milk are much more uniform than in the previous determinations, but a variation still persists. The samples to which water has been added show a regular decrease in conductivity as the amount of water added increases. A search of the literature on electrical conductivity shows that while the size and distance of the electrodes and the form of the cell are discussed, there is failure to make

TABLE IV.

TESTS USING SPECIAL CELL (No. 3).

Temperature, 28° C.

Sample of Milk.	No.	Conductivity.
Grade A (Sealect), 1 day after pasteurization	1	0.00546
Raw herd milk from fresh cows	2	0.00540
No. 2, Pasteurized at 67 to 72 C. (½ hr.) 4 hrs. after pasteurization	3	0.00541
Grade B, pasteurized (Borden's)	4	0.00563
Same, another sample	5	0.00559
Same, another sample	6	0.00559
Grade B, pasteurized (Sheffield)	7	0.00544
Grade B, pasteurized (Clover Farm)	8	0.00569
Same, another sample	9	0.00566
Grade B, pasteurized (Mutual-McDermott)	10	0.00563
No. 4 + 2 per cent. distilled water	11	0.00546
No. 4 + 5 per cent. distilled water	12	0.00537
No. 4 + 10 per cent. distilled water	13	0.00512
No. 7 + 2 per cent. distilled water	14	0.00536
No. 7 + 5 per cent. distilled water	15	0.00523
No. 8 + 5 per cent. distilled water	16	0.00541
Grade B collected from shop	17	0.00539
	18	0.00541
	19	0.00550
	20	0.00560
	21	0.00561
	22	0.00555
No. 22 repeated after 5 minutes	23	0.00554

TABLE IV—*Continued*

Sample of Milk.	No.	Conductivity
Grade B collected from shop	24	0.00558
	25	0.00577
	26	0.00579
	27	0.00535
	28	0.00558
No. 27 repeated	29	0.00536
Grade B collected from shop	30	0.00560
	31	0.00575
	32	0.00554
	33	0.00567
	34	0.00566
	35	0.00563
	36	0.00564
	37	0.00563
	38	0.00577
	39	0.00554
	40	0.00537
	41	0.00546
Grade A collected from shop	42	0.00555
	43	0.00552
	44	0.00532
	45	0.00534
	46	0.00535
	47	0.00530
	48	0.00548
Grade A, mixed samples, 1st bottle	49	0.00652
2d bottle	50	0.00633
Authentic sample	51	0.00678
No. 51, second reading	52	0.00682
Authentic sample	53	0.00450
	54	0.00643
	55	0.00843
No. 53 + 7 per cent. water	56	0.00435
Authentic sample unpasteurized	57	0.00555
Milk from 3 or 4 mixed breed cows	58	0.00552
Same as above after 10 minutes	59	0.00565
No. 59 after 40 minutes	60	0.00569
Same as No. 57	61	0.00575
	62	0.00551
No. 62, second reading	63	0.00545

mention of the error introduced by using varying amounts of solution. This phenomenon is being made the subject of a separate investigation.

As a final effort to determine the cause of the above variations in the whole milk a special cell was constructed as shown in the accompanying drawing. This cell has the advantage that the electrodes are sealed in a permanent position, a constant distance apart, and with the same amount of liquid above and below them.

The results in Table IV are most unsatisfactory, in that a wide discrepancy in conductivity exists even in authentic samples. It was then decided to separate the milk and cream and to determine the conductivity of the skimmed milk. Through the courtesy of Messrs. Paul and Terhune of the DeLaval Separator Company, a cream separator was obtained and the samples separated. Determinations were made on whole milk, skimmed milk and cream.

The determinations in Table V were made under ideal conditions. The cell was accurately filled to the mark, the temperature kept at 28.0° C. ±0.05 and correction made for each deviation. A large number of the samples were collected under the personal supervision of department inspectors so that there could be no doubt as to their authenticity. The method of measurement was sufficiently sensitive to detect 0.5 per cent. of added water, and the fact that the cell constant did not vary was confirmed by frequent determinations, using $N/50$ potassium chloride solution. To preclude the possibility of error through improper running of the separator, a representative of the DeLaval Company separated the samples in Determinations 59 to 72.

As the milk after separation contains air bubbles, the milk was filtered to remove them. It was found that the readings before and after filtration did not vary. (Nos. 10 and 11.)

As the samples were run through the separator one after another, it was deemed advisable to determine if the samples were affected by the ones preceding. Four samples were mixed thoroughly, then divided again into 4 samples and run through the separator successively (Nos. 96 to 99). Three of these results were fairly uniform.

It will also be observed that the average of the readings on individual milks corresponds very closely with the reading after mixing Nos. 88 to 94.

With all known sources of error eliminated the readings on authentic samples still showed so wide a difference as to render the method useless.

In the freezing-point determination mentioned at the beginning of the article, the fat and protein of the milk play no part and the constant depends entirely on the concentration of the substances in solution.

In the conductivity measurements, however, the fat and protein of the milk decrease the specific conductance by decreasing the number of ions between the electrodes in just the same manner as would glass beads placed in the cell between the electrodes.

A final series of determinations (Table VI) was made on samples of milk coagulated with basic lead acetate (Horne). One hundred cubic centimeters of the milk were taken and 3 grams of the acetate accurately weighed and added, thoroughly shaken and filtered to 50 cc. Readings were then made on the clear sera.

The determinations (Table VI) made on laboratory samples 1 to 16 show a fair degree of uniformity. It will be observed from a comparison of the conductivity and the chemical analysis, that the differences in fat and total solids content bear no relation to the differences in conductivity. The sera from authentic samples 18 to 23 show a far greater variation than has been observed in any of the foregoing determinations and the results are entirely unreliable. In the authentic samples adulterated in the laboratory (24 to 29) the water was first added to the samples and 100 cc. of the adulterated milk were then taken and coagulated as described above.

These results show great differences, even at the same adulteration, and these differences are greater than those between the authentic and adulterated samples. This is probably due to an irregular absorption of the lead salt by the coagulum, which would account for the lack of uniformity in the results given, as was the case in experiments mentioned under Method I at the beginning of this paper.

TABLE V.

DETERMINATION ON WHOLE AND SKIMMED MILK AND CREAM.

Authentic Samples. From 5 cows. Milk from 4 cows in each can. Morning Milkings (1–13).
Temperature, 28° *C.*

Sample.	No.	Conductivity.
Whole Milk, Can No. 1	1	0.00561
No. 1 after separation	2	0.00590
Whole Milk, Can No. 2	3	0.00592
No. 2 after separation	4	0.00629
Whole Milk, Can No. 3	5	0.00567
No. 3 after separation	6	0.00605
Whole Milk, Can No. 4	7	0.00595
No. 4 after separation	8	0.00621
Whole Milk, Can No. 5	9	0.00555
No. 5 after separation	10	0.00594
No. 5 after separation and filtering to remove air bubbles	11	0.00595
No. 4 after separation, coagulated with rennin and filtered	12	0.00672
No. 3 after separation, coagulated with pancreatin and filtered	13	0.00643
One Holstein cow producing 16 qts. per day	14	0.00539
One Jersey cow producing 14 qts. per day	15	0.00601
Mixed Jersey and Holstein herd	16	0.00555
	17	0.00547
Holstein herd	18	0.00554
No. 14 after separation	19	0.00576
No. 15 after separation	20	0.00627
No. 16 after separation	21	0.00596
No. 17 after separation	22	0.00581
No. 18 after separation	23	0.00586
No. 18 after separation and filtration	24	0.00586
Grade B, pasteurized (Borden's)	25	0.00565
Grade B, pasteurized (Sheffield)	26	0.00543
Grade B, pasteurized (Clover Farms)	27	0.00580
Grade A, pasteurized (Locust Farms)	28	0.00573
Grade A, pasteurized (Sealect Sheffield)	29	0.00560
No. 25 after separation	30	0.00596
No. 26 after separation	31	0.00581
No. 27 after separation	32	0.00605
No. 28 after separation	33	0.00599
No. 29 after separation	34	0.00586
No. 29 coagulated with rennin and filtered	35	0.00611
No. 28 coagulated with rennin and filtered	36	0.00645
One Holstein cow	37	0.00590
One Durham cow	38	0.00538
Mixed Holstein and Durham herd	39	0.00560
	40	0.00559
	41	0.00560
No. 37 after separation	42	0.00607
No. 38 after separation	43	0.00574
No. 39 after separation	44	0.00587
No. 40 after separation	45	0.00599
No. 41 after separation	46	0.00599
	47	0.00538
1 qt. morning milk, 4 or 5 cows	48	0.00562
Mixed common herd, unpasteurized	49	0.00561
No. 47 after separation	52	0.00574
No. 48 after separation	53	0.00589
No. 49 after separation	54	0.00616
No. 50 after separation	55	0.00593
No. 51 after separation	56	0.00632
Separated cream from all samples mixed	57	0.00395
Separated milk from all samples mixed	58	0.00601
Separator run by representative of DeLaval Co. Milk from single Holstein, unpasteurized	59	0.00576
	60	0.00555
	61	0.00536
	62	0.00606
	63	0.00569
No. 59 after separation	64	0.00606
No. 60 after separation	65	0.00586
No. 61 after separation	66	0.00569
No. 62 after separation	67	0.00614
No. 63 after separation	68	0.00610
Mixed separated milk + 5 per cent separated cream	69	0.00593
+ 10 per cent. separated cream	70	0.00589
Mixed separated milk, all samples	71	0.00602
Mixed separated cream, all samples	72	0.00445
Grade A, certified, unpasteurized (Sheffield)	73	0.00554
Grade A, pasteurized (Locust Farms)	74	0.00581
Grade A, pasteurized (Sheffield, Sealect)	75	0.00573
Grade A, pasteurized (Sheffield, Sealect)	76	0.00552
Grade A, pasteurized (Mutual-McDermott)	77	0.00547
No. 73 after separation	78	0.00591
No. 74 after separation	79	0.00619
No. 75 after separation	80	0.00595
No. 76 after separation	81	0.00575
No. 77 after separation	82	0.00570
Separated cream from No. 73	83	0.00448
Separated cream from No. 74	84	0.00431
Separated cream from No. 75	85	0.00435
Separated cream from No. 76	86	0.00414
Separated cream from No. 77	87	0.00417
Grade B raw taken from cans	88	0.00585
	89	0.00592
Nos. 88 and 89 mixed	90	0.00589
Grade B raw taken from cans	91	0.00545
	92	0.00537
Nos. 91 and 92 mixed	93	0.00540
Nos. 88, 89, 91, 92 mixed	94	0.00563
No. 89 second reading	95	0.00555
Nos. 88, 89, 91, 92 mixed, after separation	96	0.00589
	97	0.00596
	98	0.00592
	99	0.00594
No. 95 after separation		

TABLE VI.

TESTS USING MILK SERA.

Temperature, 28° C.

CHEMICAL ANALYSIS.

Sample	Total Solids	Fat	No.	Conductivity
AA–223	11.82	3.3	1	0.00653
AA–226	12.01	3.4	2	0.00645
AA–227	13.18	3.6	3	0.00647
AA–228	11.94	3.4	4	0.00647
AA–230	12.06	3.5	5	0.00649
AA–232	11.90	3.6	6	0.00645
AA–233	11.78	3.5	7	0.00645
AA–234	11.94	3.4	8	0.00649
AA–235	12.13	3.5	9	0.00643
B–456	11.94	3.4	10	0.00651
B–457	12.13	3.5	11	0.00649
B–460	11.70	3.2	12	0.00649
B–461	12.38	4.0	13	0.00653
B–463	12.28	3.8	14	0.00647
2A–501	12.36	3.4	15	0.00647
2B–505	11.87	3.4	16	0.00643
2A–505				
Serum + 5 per cent. water			17	0.00653
Serum from authentic milk Grade A, raw			18	0.00682
			19	0.00659
			20	0.00667
			21	0.00682
			22	0.00657
			23	0.00667
As above + 5 per cent. water			24	0.00639
+ 5 per cent. water			25	0.00663
+ 10 per cent. water			26	0.00628
+ 10 per cent. water			27	0.00616
+ 20 per cent. water			28	0.00573
+ 20 per cent. water			29	0.00591

CONCLUSIONS.

In our investigations of the conductivity method, tried out in a most exhaustive manner, with all available modifications, we were unable to obtain any uniformity in results. It was therefore decided to abandon the method as an impractical one for the detection of added water in milk.

It is to be regretted that this method proved ineffective, as a rapid and accurate method of this sort would be of great value to the food analyst.

One of the authors, Durand, proposes at an early date to continue this research, making use of the osmotic pressure of milk, in a cell specially constructed to measure the differential osmotic pressure between milks and a standard saline solution.

THE FOLIN AND DENIS METHOD OF NITROGEN DETERMINATIONS BY DIRECT NESSLERIZATION AND ITS APPLICATION TO SPINAL FLUIDS.*

R. L. Kahn

Folin and Denis[1] have recently modified the micro method for nitrogen determinations by substituting phosphoric acid for part of the sulfuric acid ordinarily employed in the destructive digestion of the nitrogenous material and by reducing the amount of free alkali in Nessler's reagent. These changes have enabled them to Nesslerize directly the ammonia produced during the digestive process, thus eliminating the aeration procedure connected with these determinations.

In attempting to apply this method to nitrogen determinations in spinal fluids, a serious difficulty has been encountered. Folin and Denis have already directed attention to the turbidity of the Nesslerized solutions due to the presence of silicates formed by the action of phosphoric acid and heat on the glass during the destructive digestion. They advise the removal of these silicates either by centrifugation or by filtration through a small cotton plug. They say, " if the sediment obtained is mixed with a red deposit, the Nesslerization has not been successful and the determination must be discarded."

The difficulty just discussed has actually presented itself in this laboratory in every determination carried out according to the original directions. Furthermore, the standard solution showed clouding invariably indicating that the turbidity is not due to the silicates alone. In these trials, the concentrated acid mixture employed consisted of three volumes of concentrated phosphoric acid, one volume of concentrated sulfuric acid and one-fifteenth volume of 10 per cent. copper sulfate solution. The two acids were first mixed and allowed to stand over night to facilitate the precipitation of calcium. The mixture was then filtered through a dry mat of asbestos in a Buchner funnel, after which the copper sulfate solution was added.

Fifteen cubic centimeters of 10 per cent. sodium hydroxide were found to be sufficient to neutralize 1 cc. of the concentrated acid mixture and permit a surplus of 2 c.c. of alkali in the solution. This was determined in the following manner. One cubic centimeter of the concentrated acid mixture on titration with 10 per cent. sodium hydroxide, with phenolphthalein as an indicator, required 9.5 c.c. of alkali for neutralization. About three-fourths of this acidity (or 7.1 cc.) was due to phosphoric acid. As pointed out by Folin and Denis, one-third of the base combining power of the phosphoric acid is not included in the titration value. Therefore, 9.5 c.c. + 3.5 c.c., or 13 c.c., were required for the neutralization of the acid. In order to permit a surplus of 2 c.c. of 10 per cent. sodium hydroxide, 15 c.c. of the alkali were employed.

Nessler solution was prepared precisely as directed by Folin and Denis.

An attempt to ascertain the nature of the precipitate responsible for the turbidity in the standard, disclosed, by physical and chemical examination, the probable presence of calcium. The precipitate of calcium formed on mixing the concentrated phosphoric and sulfuric acids is filtered off through a mat of asbestos as stated above. Traces of calcium, however.

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* Published in Jour. Biol. Chem., 1916, 28, No. 1.

[1] Folin, O., and Denis, W.: J. Biol. Chem., 1916, xxvi, 473.

are soluble in an acid solution. When, therefore, the solution is rendered alkaline by the addition of 10 per cent. sodium hydroxide, these traces separate out and cause turbidity on Nesslerization.

Having thus been unable to prepare clear solutions for color comparisons, an attempt was made to *filter off the precipitate before Nesslerization.* The results were entirely satisfactory. Nessler solution was thus added to water-clear filtrates in each case and clear solutions for color comparisons were obtained without exception.[2]

The probable loss of ammonia during the filtration process early suggested itself. On addition of a sufficient quantity of 10 per cent. sodium hydroxide to neutralize the concentrated acid and permit a surplus of 2 c.c. of alkali, ammonia is set free in the solution. Inasmuch as ammonia is extremely volatile, it was feared that traces of it might be lost during the filtration process. It was found, however, that owing to the extreme solubility of ammonia, significant losses do not occur. This was determined in the following manner.

To a 100 c.c. volumetric flask, A, were added 1 c.c. of the concentrated acid mixture, 15 c.c. of alkali and 20 c.c. of the standard ammonium sulfate solution containing 1 mg. of nitrogen, made up to the mark, shaken and filtered. 50 c.c. of the water-clear filtrate were measured out into another 100 c.c. volumetric flask, 10 c.c. of Nessler solution added, made up to the mark and shaken. To a volumetric flask, B, were added 1 c.c. of concentrated acid, 15 c.c. of 10 per cent. alkali and without adding the standard ammonium sulfate solution, this was made up to the 100 c.c. mark, shaken and filtered as above. To 50 c.c. of this filtrate were added 10 c.c. of the standard ammonium sulfate solution, Nesslerized, made up to 100 c.c., shaken and compared on the colorimeter, with a solution of flask A. The color intensity of these two solutions was found to be identical. The free ammonia present in flask A was, apparently, completely held in solution. This experiment was repeated three times with the same results.

As a result of these findings the following modification of the direct Nesslerization method has been adopted for the determination of total and non-protein nitrogen in spinal fluids. The same principle is, of course, available in the other applications of the micro method.

Total Nitrogen.—2 c.c. of spinal fluid are pipetted into a test-tube, 1 c.c. of the phosphoric-sulfuric acid mixture added and the digestion carried out over a micro burner until the appearance of sulfuric acid fumes. The mouth of the test-tube is then covered with a watch-glass and heating continued for about a minute. The color obtained is usually straw-yellow. After permiting the test-tube to cool, the contents are rinsed quantitatively into a 100 c.c. volumetric flask, using about 60 c.c. of water in the process. 15 c.c. of 10 per cent. sodium hydroxide solution are added to neutralize the 1 c.c. of concentrated acid and permit a surplus of 2 c.c. of alkali. It is then made up to volume, shaken and filtered.

Into another 100 c.c. volumetric flask are pipetted 1 c.c. of the concentrated acid mixture, diluted, 20 c.c. of the standard ammonium sulfate solution and an amount of 10 per cent. alkali solution equal to that added to the unknown. This also is made up to volume, shaken, and filtered.

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[2] It is likely that due to a higher purity of chemicals employed in the laboratory of Folin and Denis, no precipitation occurred on addition of the 10 per cent. alkali. However, as is indicated by their statement quoted above, some determinations had to be discarded from time to time apparently on account of the red deposit mixed with the precipitate. With the modification suggested, no determination need ever be discarded as is pointed out in the text later.

Aliquot portions of these filtrates are employed for Nesslerization, the amounts used depending on the quantity of nitrogen present in the total amount; also perhaps on the type of glassware used. Thus, if the total amount of nitrogen in the digestive mixture is equivalent to about 1 mg., the Nesslerization may be completed in several ways.

Twenty-five cubic centimeters of the water-clear filtrates of both the unknown and standard may be pipetted into two 100 c.c. volumetric flasks, diluted to about 75 c.c. with water, 10 c.c. of Nessler solution added to each, made up to volume, shaken and the colors compared on the colorimeter. The standard in this case will contain 0.25 mg. of nitrogen and the calculations are made accordingly. If desired, 50 c.c. quantities of the filtrates may be pipetted into two 100 c.c. volumetric flasks, 10 c.c. of Nessler solution added to each, made up to volume and the colors compared. In this case the standard will contain 0.5 mg. of nitrogen. Then again, 25 c.c. quantities may be pipetted into 50 c.c. volumetric flasks, Nesslerized and the colors compared as above. The possibilities for error are reduced to a minimum in each case because the standard and unknown, with the exception of the destructive digestion, go through the same preparation and filtration, side by side. Furthermore, no determination need be discarded due to an error or accident during Nesslerization. There is always a sufficient quantity of water-clear filtrate on hand to repeat the process.

The importance of obtaining water-clear filtrates cannot be overemphasized. The precipitate of calcium formed on the addition of the 10 per cent. alkali is gelatinous in character (probably tertiary calcium phosphate, $Ca_3(PO_4)_2$) and traces often pass through the filter paper on the first filtration. It is sometimes necessary, therefore, to pour the filtrate back into the same funnel and refilter.

Non-Protein Nitrogen.—For the precipitation of the proteins from the blood, Folin and Denis[3] employ a freshly prepared 25 per cent. solution of glacial phosphoric acid. To 5 c.c. of blood in a 50 c.c. volumetric flask they add about 20 c.c. of water and 3 c.c. of a glacial phosphoric acid, shake vigorously for 5 minutes or more, make up to volume and filter through a dry filter paper. For nitrogen détermination they employ 10 c.c. of the filtrate.

In view of the fact that the spinal fluid used contains relatively minute amounts of protein, it seemed unnecessary to dilute this fluid for precipitation. To 5 c.c. of spinal fluid in a large sized test-tube are added quantitatively 2 c.c. of a freshly prepared 25 per cent. glacial phosphoric acid solution. The test-tube is then stoppered and either vigorously shaken for about five minutes or permitted to stand one to twenty-four hours and filtered through a small dry filter paper. For duplicate nitrogen determinations, 3 c.c. quantities of the water-clear filtrates are used for digestions. From this point the procedure employed is identical with that described for total nitrogen. In each case Nessler solution was added to water-clear filtrates and not a single determination had to be discarded.

Urea Nitrogen.—The direct Nesslerization method for urea determinations in the blood as outlined by Folin and Denis[4] was adopted for the determinations of urea in the spinal fluid, as follows:

To 5 c.c. of spinal fluid in a 100 c.c. volumetric flask are added about 5 c.c. of water and 0.1 gm. of dry urease, shaken and permitted to stand at room temperature from fifteen to twenty minutes. This is then diluted with about 50 c.c. of water, 2 c.c. of freshly prepared glacial phosphoric acid added, also 0.5 gm. of Merck's blood charcoal, and made up to volume. This is shaken from time to time and allowed to stand for forty-five minutes or more, when it is ready to be filtered. Definite portions of the water-clear filtrates are used for Nesslerization as in the cases of the total and non-protein nitrogen determinations.

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[3] Folin and Denis, J. Biol. Chem., 1916, xxvi, 491.

[4] Folin and Denis, J. Biol. Chem., 1916, xxvi, 505.

In view of the fact that the aeration method for micro nitrogen determinations is still largely employed, it may not be amiss in this connection to point out some of the more important difficulties encountered by this procedure.

The suction created by a water pump was utilized in this laboratory for the aspiration of ammonia into acid solutions. The velocity of the water was irregular, depending apparently at any given time on the quantity of water used in other parts of the building. Aerations, therefore, had to be carried on for comparatively long intervals—about 3 hours at a time. Even then, however, much of the work had to be discarded because on further aspiration into fresh absorption solutions, traces of ammonia would again and again be found to have escaped aeration.

It is generally stated in text-books that fifteen minutes of rapid aeration are sufficient to aspirate the ammonia completely into acid solutions. Whether this short period is sufficient is probably questionable. On many occasions in this laboratory (on holidays, for instance, when practically no one else in the building employed water), the rapidity of the air current was nearly as great as the apparatus would permit, and after forty minutes of such aeration, traces of unaspirated ammonia were demonstrated several times.

Another factor not sufficiently emphasized in text-books is that during a rapid aeration, when the ammonia is aspirated into a single acid tube, traces of ammonia will escape absorption.

From these findings, it appears that in agreement with the statements of various other investigators,[5] in order to insure the complete removal of ammonia and avoid any possible loss of the same: (1) a rapid air current is absolutely essential; (2) the aeration should be carried on for about an hour; (3) the ammonia should be aspirated into two acid solutions instead of one and these should be combined quantitatively before Nesslerization.

For a time, clouding on the addition of dilute Nessler solution presented some difficulty. This was overcome by diluting the Nessler solution further. When employing a standard of 0.5 or 0.25 mg. of nitrogen, it was found advisable to dilute the Nessler solution 1:8 instead of 1:5. If used immediately, this dilution did not give rise to the precipitation of mercury. During the hot weather, it was found advantageous also to cool the solutions under cold tap water before Nesslerization. This factor was found to minimize the possibilities of clouding on the addition of Nessler solution.

A series of parallel nitrogen determinations were carried out by means of the aeration and direct Nesslerization methods. In the case of total and non-protein nitrogen, the latter method, as modified in this laboratory, was employed. In the case of urea nitrogen, the method was used precisely as outlined by Folin and Denis.

The following is a tabulation of the results obtained.

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[5] Compare, for example, Davisson, B. S., Allen, E. R., and Strubblefield, B. M., J. Ind. and Eng. Chem., 1916, viii, 896.

TABLE.

NITROGEN PER 100 Cc. OF SPINAL FLUID.

No.	Direct Nesslerization Method.	Aeration Method.
Total Nitrogen.		
	mg.	*mg.*
1	31.25	31.25
2	17.75	17.69
3	18.55	18.30
4	17.50	17.47
5	37.72	37.35
6	21.90	21.90
7	22.52	22.72
Non-Protein Nitrogen.		
8	14.00	13.77
9	31.09	30.97
10	14.00	13.45
11	14.35	14.05
12	15.86	15.90
13	24.72	24.85
14	33.63	33.32
Urea Nitrogen.		
15	11.55	11.22
16	15.75	15.62
17	11.36	11.36
18	20.75	20.75
19	21.62	21.62
20	6.31	6.25

A METHOD FOR FRACTIONATING FATS AND OILS.*

ARMIN SEIDENBERG

Only a limited number of the glycerides of fats and oils have so far been isolated, and in the case of no fat or oil have all its glycerides been identified. The importance of securing a more complete knowledge of the glycerides is, however, very great both in a strictly scientific sense and also in effecting a more adequate identification of the various members of this class of substances. Owing to our lack of this knowledge the identification of individual fats or oils must depend on a number of empirical constants which, for any one substance, often vary within very wide limits. Thus. while it is ordinarily possible to identify a pure fat, the detection of even comparatively large amounts of a foreign added fat cannot always be accomplished with certainty.

VARIOUS METHODS FOR THE ISOLATION OF THE GLYCERIDES

A number of methods have been tried for separating the glycerides in fats and oils but no scheme exists, the application of which is sufficiently comprehensive. Chevreul, Krafft[1] and others tried the fractional distillation of the glycerides under *vacuo,* but according to Boemer,[2] it is possible to distil only those containing fatty acids with less than 14 carbon atoms; where glycerides are present with fatty acids containing a larger number of carbon atoms, decomposition occurs. Heise[3] isolated the first mixed glyceride, oleostearin, by precipitating the ethereal solution of mkányi fat with alcohol. Holde[4] and Okada[5] separated different fractions of fat dissolved in ether or ether-alcohol by lowering the temperature. Boemer,[6] who made some very exhaustive researches into the less soluble glycerides in beef, mutton tallow and lard, isolated these by dissolving the fat in 2 to 3 times its volume of ether, benzol or chloroform and separating out 3 to 4 fractions by lowering the temperature or by adding alcohol. Fractions within 5° C. of each other were combined and taken up in an amount of solvent which would just take all the substance into solution on warming, but which, on cooling, allowed most of it to separate out again. This was continued 20 to 30 times until no substance was left in solution. Boemer and the other investigators as well, with the exception to some extent of Hansen,[7] were not successful in isolating the more soluble glycerides. The less soluble glycerides that were secured were not obtained in amounts that even approximated quantitative proportions. Even after 32 crystallizations from ether, Duffy[8] did not succeed in obtaining an entirely pure glyceride from 2000 g. of tallow, although the substance isolated amounted to only 8 g.

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* Published in Jour. Ind. and Eng. Chem., 1917, 9, p. 855.

[1] Ber., 1903, 4343.

[2] Z. Nahr. Genussm., 14, 1907, 90.

[3] Arb. kais. Gesundh., 12, 1896, 540; 13, 302.

[4] Ber., 1901, 2402.

[5] Chem. Ztg., 32, 1908, 1199.

[6] Z. Nahr. Genussm., 14, 1907, 90; 17, 1909, 353; 25, 1913, 321.

[7] Arch. Hyg., 42, 1902, 1.

[8] Jour. Chem. Soc., 5, 1852, 199.

Comparative Solubility of Glycerides.

With very few exceptions, the usual solvents for fats and oils, such as ethyl ether, benzol, chloroform, carbon disulfide, etc., do not show any marked differences in their solvent action on the various glycerides. Tristearin and tripalmitin, although entirely soluble in ether in the warm, are somewhat less soluble in the cold—less so than other glycerides. However, there are no data based on quantitative measurements. Alcohol, to some extent, exerts a selective solubility on some of the glycerides, those of the lower fatty acids and of the unsaturated fatty acids being more soluble in alcohol than those of the saturated higher fatty acids. Thus,[9] while tributyrin and tricaproin are readily soluble in cold absolute alcohol, tripalmitin and tristearin are almost insoluble in this medium in the cold. The solubility in alcohol of all the glycerides decreases with dilution.[10] Arnold[11] found in a number of fats and oils that the iodine number of the alcohol-soluble portion was higher than that of the part that did not go into solution, showing that the glycerides containing unsaturated acids are more readily soluble in alcohol than those containing the saturated acids. Davidsohn and Wrage,[12] however, have secured some results that lead them to conclusions that do not entirely bear this out. A large amount of work on the critical temperature of dissolution by Crismer,[13] Valenta,[14] Allen,[15] Cesàro[16] and others indicates that as the temperature decreases the solubility of the glycerides becomes less in alcohol, and in other solvents as well, and that this is most marked with the glycerides of the saturated higher fatty acids. In general, it may be said that the solubility of the glycerides in alcohol, as in other solvents, decreases with an increase in the number of C-atoms of their fatty acids and that those containing unsaturated fatty acids show a somewhat greater solubility than those containing saturated fatty acids with an equal number of carbon atoms. The fatty acids, themselves, although more readily soluble, show similar relative solubilities when compared with each other. This is, of course, what is generally the case in homologous series and in compounds formed from them.

General Discussion.

On account of the very large number of glycerides and the very slight differences in solubility existing between them, it seems impossible to secure any precipitant or solvent that will separate the glycerides sharply from each other. The conditions of insolubility must be graded so finely that the very slight differences existing between the glycerides will to some extent be approximated. Neither by the very slow lowering of the temperature of a solution containing the glycerides nor by the very slow addition of a precipitant such as alcohol to their solution is it possible to attain this object with the needed refinement. In fact, in the latter case, since certain of the glycerides are distinctly soluble in alcohol, this medium would not serve to throw them out of the increased volume of solution formed by its addition. If a dilute alcohol is chosen there is even less possibility of adjusting conditions with the minute delicacy needed.

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9. Lewkowitsch, "Oils, Fats and Waxes," 5th Ed., 1, pp. 21-27.
10. Vandervelde, Bull. soc. chim. belg., 25, 1911, 210.
11. Z. Nahr. Genussm., 14, 1907, 179, 194; 16, 1908, 666.
12. Chem. Rev. d. Fette. u. Harz. Ind., 22, 1915, 2, 11.
13. Bull. soc. chim. belg., 9, 1895, 71; 9, 1896, 359; 10, 312.
14. Jour. Soc. Chem. Ind., 1884, 623.
15. Ibid., 69, 1886, 282.
16. Bull. acad. roy. med. belg., 1907, 1004.

OUTLINE OF PROPOSED METHOD.

The requirements for securing the requisite refinement in the gradation of the solubility conditions can be much more readily attained when an exactly opposite procedure is pursued, *viz.*, if from an alcohol-ether solution of the glycerides, first the ether and then the alcohol, be removed very gradually. This may be accomplished by aspirating air by means of the water pump through the solution of the glycerides in an appropriate alcohol-ether mixture. At first mainly ether with a small amount of alcohol is suctioned off, but after all the ether has been removed the alcohol continues to go. At the reduced pressure produced by the suction of the water pump the temperature of the liquid drops considerably (to about 5° C.), at which point the differences in the solubilities of the various glycerides are much accentuated. The removal of the solvents can be graded to progress with any desired measure of refinement by adjusting the degree of suction produced by the water pump. Throughout the process a very thorough agitation of the solution should be secured. In this way, by the very gradual removal of minute amounts of the solvents in the general order of their solvent action on the glycerides, accompanied by an equally gradual lowering of the temperature, the many nearly related points of comparative insolubility are approached so slowly that the separation of the various glycerides can be secured to some extent.

At first, of course, the more insoluble glycerides are thrown out of solution, but as the ether, and finally the alcohol, is aspirated off, the other glycerides, soluble in the latter medium, gradually come down as well. Naturally there is no one point at which any one glyceride is entirely precipitated. However, the largest part usually does come down within fairly narrow limits. The conditions are somewhat similar to those met with in the fractional distillation of nearly related liquids.[17] One can expect to secure a glyceride in its pure state only with repeated fractionations after having combined similar fractions. The conditions are complicated by the ready solubility of the glycerides in each other and by their occasional formation with the solvent of colloid or emulsoid solutions. It is of course necessary to regulate conditions so that the latter result is avoided. Besides ether and alcohol or ether, alcohol and water, any other combination of solvents may be chosen provided that those having the greatest solvent action are also the more volatile.

EXPERIMENTAL.

GENERAL PROCEDURE.—Since by the methods heretofore proposed it has been possible to isolate only the less soluble glycerides of fats and oils, the writer undertook the isolation of the more soluble glycerides in a sample of tallow according to the method described. To 100 grams tallow were added 1000 c.c. of a mixture of 50 parts ether and 50 parts absolute alcohol, warmed slightly and then allowed to cool. A heavy white residue formed which was filtered off as Residue A. Through the filtrate air was aspirated by the suction of the water pump. The solution became turbid at about 650 c.c., but suction was continued until a heavy precipitate had formed, which was filtered off as Residue B. The filtrate from this amounted to about 450 c.c. and the suction on it was continued until it had been reduced to 200 c.c. On being allowed to stand, the turbid liquid separated into two liquid layers. The lower one, which had a very decided yellow color, was

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17. Young, "Fractional Distillation," 1903, Chapter 7, p. 114.

separated by means of a separatory funnel as Residue C. Suction on the remainder was continued until it was reduced to 100 c.c. when it became only slightly turbid. It was evaporated on the water bath, but as only a very slight residue was left this was discarded.

Each one of the above residues was now again taken up in a mixture of ether and alcohol: Residue A in a 500 c.c. mixture of 10 parts absolute alcohol and 90 parts ether (since only small amounts of alcohol-soluble glycerides were present); Residue B in 300 c.c. of a mixture of 40 parts absolute alcohol and 60 parts ether (since this contained a larger proportion of the alcohol-soluble glycerides); and Residue C (after evaporating the alcohol and ether with which it had previously formed a solution), in a 100 c.c. mixture of 40 parts 95 per cent. alcohol, 10 parts water and 50 parts ether (since here the amount of the less soluble glycerides was slight). Through each of these solutions air was now aspirated and in each case 3 to 4 fractions were secured. The melting points of these fractions were taken and those within 5° C. of each other were combined and dissolved again in mixtures of alcohol and ether or of alcohol, water and ether proportioned according to the number, quantity and solubility of the glycerides which could be expected to be present. These were again fractionated and recombined as above until the various fractions from any one solution gave identical melting points. The separation of the solid residue was usually accomplished by pouring both residue and liquid upon a filter paper and drawing off the liquid by means of suction, stirring toward the end with a glass rod so that as much as possible of it was expressed.

Most of the more soluble glycerides would of course be contained in Residue C, but some would also be in the end fractions from Residues A and B. Residue A was fractionated into four parts having, respectively, the melting points, 57°, 56°, 55° and 44° C. Residue B was fractionated into three parts having, respectively, the melting points 50°, 45° and 35° C. On combining the similar end fractions from these, other fractions with the following melting points were secured: 52, 45, 40, 38, 34, 32, 30, 27, 25, 24, 22, 18 and 15° C. These were again recombined and refractionated and those having a melting point below 25° C. combined with Residue C, which was now fractionated according to the procedure outlined, usually from a mixture consisting of either 50 parts ether and 50 parts 95 per cent. alcohol or 50 parts ether, 40 parts 95 per cent. alcohol and 10 parts water. Those fractions that were nearly related in melting point were combined and refractionated. This was continued until after fractionating the lower melting point fractions eleven times and the higher melting point fractions seven times two distinct yields were secured which seemed on further fractionation to be pure compounds.

To identify a glyceride the constants usually deemed necessary are: melting point, saponification number, neutralization number of the fatty acids (average molecular weight) and the iodine number. In order to obviate the possibility that the substance under examination is not a mixture of several glycerides, it is necessary to determine one of the constants, preferably the melting point, on several fractions. If all the fractions have the same constant the substance may, according to Boemer,[18] be considered a pure glyceride. Another indication that the substance is not a mixture can be obtained by observing whether all or practically all of it is precipitated at one point during the suction instead of gradually.

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[18] Z. Nahr. Genussm., 14 (1907), 90.

HIGHER MELTING POINT YIELD.

The higher melting point yield from Residue C amounted to only 0.98 g. and gave the following constants:

	Observed Determinations.			Calculated or Known Constants for Oleodistearin.
	1.	2.	Average.	
Melting point (corrected)	44.0	44.4	44.2	44–44.6
Iodine number	27.0	26.7	26.9	28.6
Saponification number	187.4	....	187.4	189.5
Neutralization number	197.7	....	197.7	197.9

On fractionating the material into three fractions these were found to have melting points (corr.): 44.6, 44.0, 44.4° C. The substance, although the results for the iodine number were slightly low, may be regarded as the glyceride oleodistearin.

This glyceride has been isolated by Heise[19] from mkányi fat and kokum butter. According to Fritzweiler,[20] it also occurs in cocoa butter, and according to Klimont,[21] in Borneo tallow. It has, however, not as yet been isolated from beef or mutton tallow. In appearance it is a white solid substance forming small crystals.

LOWER MELTING POINT YIELD.

The lower melting point yield secured from Residue C amounted to approximately 20.6 g. and gave the following constants:

	Observed Determinations.			Calculated or Known Constants for Dioleopalmitin.
	1.	2.	Average.	
Melting point	(Indistinct)	(Indistinct)	10 to 12	....
Iodine number	51.2	50.6	50.9	59.2
Saponification number	197.6	196.0	196.8	196.1
Neutralization number	202.7	204.8	203.8	205.2

Since the melting point was not sharp the iodine number was determined on four fractions of this material with the following results: 50.7°, 51.8°, 50.6°, 50.3°. The agreement in itself is sufficiently satisfactory to stamp the material as a pure glyceride. However, there is a considerable deviation in this iodine number from that required by theory for dioleopalmitin, to which the other constants correspond quite closely and to which also the observed iodine numbers approach nearer than to any of the other glycerides that can occur in tallow except dioleostearin. The glycerides present in any fat are dependent, of course, upon the fatty acids to be found in the fat which in this instance are stearic, palmitic and oleic acids. This

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19. Z. Nahr. Genussm., 14 (1907), 90.
20. Arb. kais. Gesundh., 1902, 371.
21. Monatsh, 1904, 929; 1905, 563.

deviation in the iodine number may be explained on one of two grounds. It may be due to the admixture of another glyceride with a lower iodine number. However, the number of fractions having practically identical constants would hardly justify this assumption. On the other hand, it is possible that the long-continued passage of air through the solutions containing this substance or its frequent exposure to heat when the solvent was evaporated off may have affected the unsaturated bond. This is borne out to some extent in the literature and may also explain the indistinct melting point. Thus Lewkowitsch[22] reports oxidation of the fatty acids on blowing air through various fats and oils at an elevated temperature and Fahrion[23] states that the fatty acids are affected by heat. The error due to this cause may possibly be avoided by removing the oxygen from the air before aspirating it through the solution. In order to obviate all possibility of confusion with dioleostearin the saturated fatty acid of the substance was fractionated from an appropriate alcohol-ether mixture according to the general procedure outlined for the glycerides. In this way a saturated fatty acid was secured, three fractions of which had the melting points (corr.): 62.0°, 61.8°, 62.2° C. The melting point of palmitic acid is[24] 62.6° C.

It may therefore be stated that the substance was dioleopalmitin which had been affected to some extent so that it did not add on the theoretical quantity of iodine. At room temperature it was a yellowish liquid, but on solidifying became almost white in color.

Besides the two glycerides which were isolated from Residue C, some material remained. Although this was not identified, it probably consisted of a mixture of the two glycerides that were isolated. There was no evidence of the presence of triolein and therefore the dioleopalmitin may, in all probability, be considered the glyceride present in tallow which is most soluble in ordinary solvents.

Olein-Free Residue.

No attempt was made to fractionate completely Residues A and B. It is, however, of interest to add that it was possible to secure about 13 g. of olein-free glycerides from these two residues after four re-fractionations. The iodine number of this material was zero and it showed only a very faint flame test according to Kreis and Hafner.[25] These two investigators[26] considered it impossible to secure an olein-free glyceride by crystallization from alcohol, ether, chloroform or other solvents. Boemer[27] secured olein-free substance only after numerous crystallizations and then only in comparatively small amounts.

Detection of Tallow in Butter Fat.

The readiness with which this comparatively large amount of olein-free substance was secured led the writer to make comparisons between the different quantities that could be isolated in this way from various other fats. From the results obtained it was shown that by standardizing conditions the method could be adopted to detect the addition of certain foreign fats to butter fat in quantities that could not be ascertained by

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22. Analyst, (1899), 322.
23. Chem.-Ztg., 31 (1907), 434.
24. Lewkowitsch, "Oils, Fats and Waxes," 5th Ed., 1, p. 158.
25. Z. Nahr. Genussm., 7 (1904), 655.
26. Ber., 36 (1903), 1123.

means of the usual analytical constants. Thus to 5 g. of butter fat was added a mixture of 80 parts ether and 20 parts 95 per cent. alcohol, so that the combined volume amounted to 75 c.c.. This was reduced by suction to 55 c.c. and the resulting precipitate filtered off. This precipitate was again dissolved in a mixture of 70 parts ether and 30 parts 95 per cent. alcohol to a total volume of 50 c.c. By suction this was reduced to 30 c.c., the resulting precipitate again filtered off and weighed. It amounted to 3.8 per cent. When the same sample of butter fat containing 20 per cent. added tallow was subjected to this procedure a residue amounting to 9.9 per cent. was obtained. The Reichert-Meissl number, which is the constant most characteristic for butter fat, was in the above instances, respectively 36.8 for the pure butter fat and 26.9 for the butter fat containing 20 per cent. added tallow. The latter figure is still well above the minimum for butter fat, which runs as low as 22 and at times is even less.[28] In the case of the residues the difference caused by the 20 per cent added tallow amounts to 106 per cent. of the original amount; in the case of the Reichert-Meissl number it amounts to only 27 per cent. It is the intention of the writer to make further investigations of the amount of residue to be secured under standardized conditions from representative samples of butter of undoubted purity.

SUMMARY.

The proposed method consists, essentially, of dissolving a fat or oil in two or more solvents, one of which is more volatile and exerts the greater solvent action on the glycerides. This solvent is first very gradually removed by aspirating air through the solution, after which the remaining solvent is removed in the same way. This is accompanied by a considerable lowering of the temperature. Due to the slow decrease in temperature and to the gradual removal of the solvents until none is left, the various glycerides by degrees come out of solution in the order of their insolubility in the solution remaining. At the same time it must be pointed out that all of any one glyceride does not come down entirely at a definite concentration and that in order to secure a chemically pure product the fractionation after combining like fractions must be repeated as often as necessary. The substance may then be secured in amounts approximating its quantitative presence in the fat. The theoretical conditions resemble in many respects those existing during the fractional distillation of a mixture of liquids. Two of the more soluble glycerides of tallow were isolated by this method and it was demonstrated that a wider and more accurate knowledge of the glycerides in the various fats and oils would prove valuable not only from a scientific standpoint but also in the detection and identification of adulterants.

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[27] Ber., 36 (1903), 1123.

[28] Lewkowitsch, "Oils, Fats and Waxes," 5th Ed., 2, p. 823.

THE INCIDENCE OF TYPHOID CARRIERS IN A STATE INSANE ASYLUM.*

Charles Krumwiede, Jr., and Elbert M. Somers.

In September and October, 1907, there were some cases of typhoid fever in the Brooklyn State Hospital. At this time, Drs. Smith and Agnew of this institution sent to Miss W. Carey Noble of the Bureau of Laboratories the stools of all the convalescent cases. Continued examinations of these cases showed that two women (E. O. and A. M.), had become chronic bacillus carriers. In 1912, there were again a few cases of typhoid fever and from these developed another chronic carrier (M. R.). Again, in 1915, a few inmates contracted the disease.

As previous reports have shown a tendency toward a high carrier rate in asylums, we thought it worth while because of this history of institutional fever to cooperate in a survey to determine the incidence of typhoid carriers. Under the direction of one of us, the institution was listed by wards and ward residence since 1907 indicated for each inmate. Specimens of feces were collected and brought to the laboratory as soon as possible after evacuation and were examined there for the bacillus typhosus.† All the inmates in the female wards were examined, yielding the following results:

Wards 1 *and* 2—Feb., 1916, population 91. One resident had had typhoid in Ward 9 in 1907. No cases of typhoid had developed since in these wards. No carriers were found.

Wards 3 *and* 4—March, 1916, population 65. Two cases of typhoid had occurred in this ward in 1907, both patients becoming carriers (E. O. and A. M. mentioned above). Two additional carriers (M. C. and H. G.) were found at the time of our survey, both without any history of typhoid. M. R. chronic carrier (mentioned above), who developed typhoid in Ward 9 in 1912, was transferred to this ward later in the same year, and had died here in September, 1915.

Ward 5—April, 1916, population 80. One case of typhoid had developed in 1907. Another patient had had typhoid in Ward 9 in 1912. No carriers were found.

Ward 6—April, 1916, population 132. No cases of typhoid had developed in this ward. No carriers were found.

Ward 8—April, 1916, population 72. No cases of typhoid had developed in this ward. Three residents gave typhoid histories; two before admission to the hospital, one in 1907, in Ward 9. Examinations negative. Two carriers (J. L. and M. W.) were found, both without any history of typhoid fever.

Ward 9—May, 1916, population 76. Eight cases of typhoid fever had developed in this ward: two in 1907, four in 1912, two in 1915. (Three of these now resident in other wards, and noted above.) One 1912 case (M. R.) became a chronic carrier and was transferred to Ward 3 (q. v.). Examinations of the four remaining former typhoid patients were negative. One typhoid carrier was found (M. B.) who denies a history of typhoid fever.

The changes in ward residence of the carriers and of other carriers, then unrecognized, may have led to contact infection and the development of the carrier state without recognizable symptoms of typhoid infection.

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* Published in Jour. Am. Med. Ass., 1918, 71, p. 2131.

† We are indebted to Misses Ann Kuttner and Elsie Schumm for the performance of most of these examinations.

TABULATION OF CARRIERS.

SURVEY 1916.

FORMER CARRIERS.

Had been residents in various wards	E. O. A. M. M. R.	Carrier from typhoid in 1907. Carrier from typhoid in 1907. Carrier from typhoid in 1912.

NEW CARRIERS FOUND.

Ward 3 and 4......................	M. C.	
Ward 3 and 4......................	H. G.	
Ward 8..........................	J. L.	
Ward 8..........................	M. W.	
Ward 9..........................	M. B.	

It will be seen from this summary that, in a total population of 516 female patients, the survey yielded five chronic carriers in addition to three previously known. Of this latter group of three, all continued to excrete bacilli after having had typhoid fever, two in 1907 and one in 1912. Of the other five, not one gives a history of typhoid fever. Two much reliance cannot be placed on negative histories of typhoid fever, especially from patients of this type, as the symptoms of a mild infection may have been overlooked.

In spite of this, however, we believe that it is not an unreasonable assumption that some of these patients on coming in contact with cases of carriers, ingested the bacilli and becoming normal or contact carriers developed into chronic carriers later. That is, their natural immunity prevented the development of a generalized disease; but in spite of this resistance, the bacilli after multiplying in the intestines may have invaded the body proper in small numbers and, through the lymphatic and vascular systems, may have reached the liver and gall bladder. Here they localized and gave rise to chronic inflammation with a resulting chronic carrier state.

The striking thing in this investigation is the large number of carriers, 8 among 516 persons or 15.5 per thousand. This rate is higher than that encountered usually in institutions and much higher than among the general population. The unusually high incidence is shown by applying the rate to the female population of New York State, over twenty-five years of age. This would give the extraordinary result of 45,000 carriers.

In view of these results in addition to the frequency of institutional typhoid fever, we do not believe that any one would question the advisability of vaccinating against typhoid fever all inmates of insane asylums. Unfortunately, however, this procedure is left to the discretion of the institutional authorities and is not carried out as generally as it should be. Since a considerable amount of institutional typhoid fever due to the failure to vaccinate has come to our notice during the last few years, we feel that vaccination should be mandatory not only in asylums but in all custodial institutions and a regulation to this effect should be added to the Sanitary Codes of all Health Departments.

A TYPHOID-CARRIER DISCHARGED DURING CONVALESCENSE AFTER THREE NEGATIVE FECAL EXAMINATIONS. TYPHOID HISTORY IN HER FAMILY.*

Charles Krumwiede, Jr., and Morris L. Ogan

It is undoubtedly a valuable requirement that the feces and urine of a typhoid patient should, prior to discharge, be found negative on bacteriological examination. Such negative examinations, however, are not conclusive evidence that the excretion of bacilli has ceased or if it has ceased, that excretion may not be resumed. During convalescence the excretion may be very irregular and a negative result may be due to the fact that the number of typhoid bacilli present is so small that they cannot be isolated by our present methods. Furthermore, actual absence of bacilli from the feces would not exclude the presence of developing biliary lesions which would later discharge bacilli into the intestinal tract. Although these facts are generally known, it is seldom that we encounter such a striking illustration of their importance as the one here reported.

On April 28th, 1914, we were notified of the death of Mrs. Las. An autopsy was obtained and the post-mortem findings verified the clinical diagnosis of typhoid fever. The typhoid fever was complicated by the abortion of a six months' fetus. Investigation revealed an interesting family history of typhoid, showing the importance of contact infection. It also revealed the presence in the family of a chronic carrier.

Four related families were found involved. Of a total of 37 persons 12 had developed typhoid fever. Three of the families "C," "G," and "Lib." are related in that the mothers are sisters. "Mrs. Las." was the daughter of Mrs. "C."

The cases of typhoid fever in the order of their onset were as follows:

"C" family—Mike, onset Sept. 3, 1913. Age, 17 years.
"C" family—Mrs. C., onset Oct. 3, 1913. Age, 54 years.
"C" family—Julia, onset Oct. 3, 1913, or possibly before. Age, 15 years.
"C" family—George, onset Oct. 13, 1913, probable typhoid. Age, 25 years.
"G" family—Angelina, onset Oct. 8, 1913. Age, 2 years.
"G" family—Rocco, onset Oct. 11, 1913. Age, 6 years.
"Lib." family—Julia, onset Oct. 14, 1913. Age, 15 years.
"Las." family—Rocco, onset March 11, 1914. Age, 10 years.
"Las." family—Daniel, onset March 20, 1914. Age, 12 years.
"Las." family—Louis, onset March 20, 1914. Age, 8 years.
"Las." family—Therese, onset March 20, 1914. Age, 4 years.
"Las." family—Mrs., onset April 1, 1914—Died. Age, 30 years.

The probable sequence† of infection is as follows:

Mrs. C. and Julia C. were probably infected while nursing Mike, who did not enter the hospital until late in the course of the disease, when he was sent because of the development of a peri-renal abscess. Since Mike did not enter the hospital until October 11th, he (not the mother and sister taken ill Oct. 3), was probably the source of infection for George, too.

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* Published in Public Health Reports, U. S. Public Health Service, 1918, 33, p. 99.
† Based on statements of family.

The members of the "G" and "Lib." family visited the "C" family during the illness of Mike and may easily have been infected from this source, especially considering the cramped quarters that the family occupied.

With the developing pregnancy of Mrs. Las., the mother, Mrs. "C." was called upon for help in the household work and came in more frequent contact with the members of the Las. family. Although Mrs. "C" had been discharged from the hospital only after three negative examinations of her feces, she was found to be a carrier when examined in May, 1914. She is still excreting typhoid bacilli at the present time, although remittently. A remission of nearly six months has occurred while under our observation. There can be little doubt that she infected the five members of the Las. family. In addition, one friend of the family was also thus infected.

The data presented give striking argument for the Department procedure of enforced hospitalization of typhoid fever cases whenever the household conditions do not allow of complete isolation with a nursing attendant not engaged in other household activities. Had the first case in the above family been removed, we should have prevented the development of twelve subsequent cases one of which, having become a chronic carrier, remains a permanent menace to the community.

Negative examinations for discharge are a safeguard only in so far as they prevent the return of convalescents to their homes while still excreting typhoid bacilli in appreciable numbers. The history of the above carrier indicates the value of a prolonged period of observation of those convalescents engaged in occupations where they handle foodstuffs. According to present Department procedure, such persons are kept under observation and re-examined over a period of six months.

FECAL EXAMINATIONS OF A REGIMENT INFECTED WITH BACILLUS PARATYPHOSUS A, WITH SPECIAL REFERENCE TO NORMAL CARRIERS.*

Charles Krumwiede, Jr.

The Fourteenth Infantry, N. G. N. Y., returned last fall from the Mexican border badly infected with paratyphoid (A type). Dr. Charles W. Berry has reported the epidemiology of this outbreak.[1] The onset of paratyphoid fever was foreshadowed and accompanied by many cases of enteritis with diarrhea. As far as we know, however, Bacillus paratyphosus A, in contrast to other members of the paratyphoid-enteritidis group, does not cause acute enteritis; paratyphoid A infections, clinically and epidemiologically, are closely allied to typhoid fever. The close resemblance of this outbreak to water-borne typhoid epidemics, which are frequently accompanied by outbreaks of diarrhea, indicates strongly that it was water-borne.

In all, about 215 cases were diagnosed among 1000 men; the total incidence of the disease was probably higher. Because of this high incidence, a general fecal examination was undertaken after the return of the regiment to the New York armory, preliminary to discharge. Altogether, 843 men were examined; this paper presents an analysis of the results of these examinations. The specimens were collected under the supervision of Captain Charles W. Berry, M. D., who also supplied the histories. Special cases were investigated further by Dr. E. F. Marsh. The bacteriologic examinations were made by Ann Kuttner, Elsie Schumm, and Margaret Kelley; the final identification of the strain isolated was made by Lawrence A. Kohn.

The number of cases examined is too few to establish the incidence of excretion of bacilli at different periods after infection, but the results are suggestive. The percentage of positive findings during the first week is much higher than in typhoid fever. During the second week, it about equals the highest percentage (53 per cent.) which has been recorded for typhoid (Gaeghtens and Brüchner[2]). In the third and fourth weeks, the percentage falls, whereas all investigators have found an increased excretion after the first week of typhoid fever which reaches its height, as a rule, during the third week. This marked difference is probably correlated with differences in the intensity and extent of intestinal lesions in the two diseases and not accidental, due to the small number of cases examined. It has been possible to repeat the examinations on only a few of these men, so that I can give no data on the incidence of chronic convalescent carriers. As individual case histories are not available, the results cannot be analyzed in relation to the time of disappearance of the fever, and as the duration and intensity of the disease varied so considerably, an average febrile period cannot be used. Besides the cases tabulated, one man with positive fecal results developed a pyonephrosis 12 days later. He had not been ill previously and, unfortunately, it was not possible to determine whether this condition was due to a renal localization of B. paratyphosus A.

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* Published in Jour. Inf. Dis., 1917, 21, p. 141.

[1] Med. Rec., New York, 1917, 91, p. 135.

[2] Cited by Ledingham and Arkwright, The Carrier Problem in Infectious Diseases, 1912, p. 10.

TABLE 1.

Result of Examination; Cases of Paratyphoid Fever.

Time of Examination	Examined (No.)	Positive (Per Cent.)
Incubation (period of one week before onset)	13	15
At onset or during first week	6	83
During second week	2	50
During third week	9	33
During fourth week	10	20
During fifth week	12	15
During sixth and seventh week	5	20
Total	57	

Exclusive of the cases cited, there remains a total of 786 men. Of these, 32 or 4 per cent. gave positive fecal results. Twenty-one were reexamined 10 to 12 days later and 9 were again positive. The histories of these men were carefully investigated, and no record of illness before or after the time of examination was found. Although the absence of any history of illness is not conclusive evidence that none of them had had mild transient symptoms, it is fairly conclusive for the following reasons: At the border the camp conditions were rigorous and the temperature high, and the added strain of drills and forced marches makes it unlikely that any man who was even slightly ill would not have reported to the medical officer. The men were from urban life and occupations, and were not hardened to the strain of actual field service. As soon as the presence of a febrile epidemic was recognized, the medical supervision became rigid, and a general thermometer inspection was instituted. After the return of the regiment it is unlikely that any illness would have been overlooked, as the men were closely observed until the time of discharge. Subsequent to discharge, which took place after all the fecal examinations were completed, no case of illness was reported in this group of men.

It therefore seems justifiable to put these men in the class of normal carriers, that is, in the group of intestinal carriers who without evidence of disease, harbor and excrete bacilli for a variable, but usually short, length of time. As such, the percentage (4 per cent.) is very high. Doubtless, it is directly proportional to the high degree of exposure to infection and to the relatively low virulence of the infecting type, which, in spite of intestinal multiplication, did not go on to general invasion. It is hardly necessary to emphasize the important rôle these men would have played in the spread of infection had they remained unrecognized, which would have been the case had general fecal examinations not been instituted.

The methods employed in the examinations were the use of brilliant-green agar previously reported,[3] the tentative agglutination from the plate by the macroscopic slide method, using a paratyphoid A serum, and the isolation of positive colonies with the Russell medium, followed by final cultural and agglutinative identification. None of the strains isolated fermented xylose, and all were agglutinated by the type serum. As a strain isolated from a member of this regiment was at our disposal at the beginning of the investigations, the brilliant-green medium was standardized directly against the infecting organism. From 0.35 to 0.5 c.c. of a 0.1 per cent. solution of brilliant green to 100 c.c. of agar gave good results, the amount applicable varying with different batches of agar. Because of the relatively marked resistance of the type and the large volume of work, only one strength of dye was used, and the high concentration of brilliant green made heavy inoculation possible. Two dye and two Endo plates were used for nearly all samples. The comparative results, where recorded, were: dye agar, 48 positive, and Endo plates, 25 positive, or 52 per cent. of total positive findings. Undoubtedly, more elaborate technic would have increased, to some extent, the total number of positive findings.

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[3] Krumwiede, Pratt and McWilliams. Jour. Infect. Dis., 1916, 18, p. 1.

SUMMARY.

Examination of the stools of 57 men with paratyphoid fever gave the highest percentage of positive results (83 per cent.) during the first week of the disease.

In a series of 786 men exposed to infection, 4 per cent. of carriers of B. paratyphosus A had no history of a paratyphoid infection and may be classed as normal carriers. The duration of this condition was probably short in most instances.

The use of a selective plating medium for isolation resulted in a marked increase in positive results.

DETERMINATION OF THE TYPE OF PNEUMOCOCCUS IN THE SPUTUM OF LOBAR PNEUMONIA.

A Rapid, Simple Method.*

Charles Krumwiede, Jr. and Eugenia Valentine

As a result of the observation that a boiling alkaline hypochlorite solution would not destroy the precipitable substance of bacteria, we developed a rapid method for the preparation of a concentrated precipitin antigen from bacteria.[1] The same method was found to be applicable to the preparation of a precipitin antigen from pneumonia sputum. In an appreciable proportion of specimens the type of pneumococcus present could be identified by this method in from one half to one hour after the receipt of the sputum. More recently, Mitchell and Muns[2] have reported a less complicated method for the preparation of an antigen from sputum. Their reports, as well as the results of our attempts to shorten the time in carrying out the mouse test according to the method of Blake,[3] indicated that many sputums are comparatively rich in soluble antigen. This being so, the solution of the pneumococci in the sputum, the object of the alkaline hypochlorite method, would be unnecessary.

Although the sand method of Mitchell and Muns is comparatively simple, we were certain, because of the facts given, that a still simpler method could be devised. One of the steps in the hypochlorite method involved the removal of the coagulable albumins. We had noted that the specimens yielding positive results were usually very rich in coagulable albumins, which one would expect to be the case in an inflammatory exudate of pulmonary origin. In fact, the presence of albumin has been used for diagnostic purposes, especially for the examination of sputums from pulmonary tuberculosis. A trial showed that sputums having the appearance of being actually raised from the lung coagulated more or less firmly when heated to 100 C. On this basis we evolved the following rapid method of preparing a precipitin antigen directly from sputum:

Technic.

From 3 to 10 c.c. of the sputum, depending on the amount available, is poured from the sputum container into a test tube. This is placed in boiling water for several minutes or longer until a more or less firm coagulum results, which will occur if the specimen is a suitable one. The coagulum is then broken up with a heavy platinum wire or glass rod, and saline is added. Just enough saline should be added so that, on subsequent centrifuging, there will be sufficient fluid to carry out the test. If too much is added, the resulting antigen may be too dilute. In some instances little or no saline is necessary, as sufficient fluid separates from the coagulum.

After the addition of the saline, the tube is again placed in boiling water for a few minutes to extract the soluble antigen from the coagulum, the tube being shaken several times during the heating. The broken clot is then thrown down by centrifuge, and the clear supernatant fluid used for the test. To hasten the appearance of the reaction and to obtain a reaction even should the antigen be dilute, we layer the antigen over the "type" serums, using the latter undiluted. Two-tenths c.c. of the three "type" serums are placed each in a narrow test tube, and the antigen added from a capillary tube with a rubber teat. If the tubes containing the serum are tilted and the antigen dropped slowly on the side of the tube just above the serum, no difficulty will be encountered in obtaining sharp layers, as the undiluted serum is sufficiently higher in its specific gravity. The tubes are then placed in the water bath at from 50 to 55 C. and observed after several minutes.

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* Published in Jour. Am. Med. Ass., 1918, 70, p. 513.

1. Krumwiede, Charles, Jr., and Noble, W. Carey: Jour. Immunol, 1918, iii, 1.
2. Mitchell, O. W. H., and Muns, W. E.: Jour. Med. Research, 1917, 37, 339.
3. Blake, F. G.: Jour. Exper. Med., 1917, 26, 67.

If a fixed type be present in the sputum, and should the sputum be rich in antigen, a definite contact ring is seen in the tube containing the homologous serum. With sputums less rich in antigen, the ring may develop more slowly, and it will be less marked. Some experience is necessary in detecting the less marked contact rings and in differentiating them from an apparent ring; this may be confusing, if one of the serums is darker in color, thus giving a sharper contrast, with the supernatant antigen. The true ring is more or less opaque, and this quality can be seen by tilting the tubes and looking at the area of contact against a dark background, for example, the lower edge of a dark shade raised to just above the level of the eyes. The advantage of the ring test is that a ring may be evident at once, whereas definite clouding or visible precipitate may appear only after longer incubation, or may be so slight even after an hour's incubation as to leave one in doubt. It is well to shake the tubes after twenty minutes, as many of the specimens will show definite clouding or precipitate either at once or on further incubation, thus checking the ring reading. All of the observations given in the accompanying table were read within one-half hour, and the majority were evident in less than ten minutes.

Results.

The table gives the results thus far obtained. The quality of the sputum is noted and also the degree of coagulation, for it is evident on comparing these data with the results that the success of the test depends on the quality of the sputum submitted. The specimens examined were the routine samples submitted to the laboratory by private physicians, and no special care was taken in the selection of the sample. The results with the sand method of Mitchell and Muns, the ring test being used, are given whenever sufficient material was available for duplicate tests. A few examinations by the method of Avery[4] are also given because they are based on the results of broth cultures incubated from five to eighteen hours. This method, therefore, may be employed as a check method, even though the tubes are inoculated too late for examination on the same day. Further observations are necessary, however, as to the possible disturbing influence of overgrowth by other organisms after a period of incubation longer than five hours, which might lead to a negative result. Sterile citrated horse blood was employed in place of the defibrinated rabbit blood advised by Avery. The layer method was not used with the Avery method, as the broth does not layer well. The results were finally checked by mouse inoculation or the isolation of pure cultures directly from the sputum. All the Group IV results were checked by mouse inoculation. to exclude the possibility of the presence of small numbers of pneumococci of the fixed types.

The coagulation method failed in four instances in which fixed types were present, but in each case the sputum was an unsatisfactory sample, which was evident on inspection, and coagulation also was absent. If coagulation does not occur, there is little use in proceeding with the method. In some instances in which coagulation has been slight and the extract opalescent, dilution of the extract has cleared this sufficiently so that a ring reaction could be seen. In these instances, naturally, the content of antigen in the sputum was large. We have attempted to coagulate the mucoid types of sputum by adding egg albumin. This has failed, for on subsequent extraction with saline the mucus goes into solution, and a clear antigen cannot be obtained.

We have obtained in this series somewhat more successful results with the coagulation method than with the sand method. To our mind the advantages of the former are the lessened labor in performing the test,

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[4] Avery, O. T.: Determination of Types of Pneumococcus in Lobar Pneumonia, The Journal A. M. A., Jan. 5, 1918, p. 17.

RESULTS.*

No.	Quality of Sputum.	Coagulation.	Coagulation Method.		Sand Method.		Avery Method.		Result of Final Check Test: Type or Group.
			Type.	Degree of Reaction.	Type.	Degree of Reaction.	Type.	Degree of Reaction.	
1	Good	+	III	+	III	+	..	..	III
2	Good	+	III	+	III	+	..	..	III
3	Good	+	I	+	..	..	..	..	I
4	Good	+	III	+	III	+	..	..	III
5	Mucoid	—	—	—	—	—	..	..	I
6	Good	+	II	+	II	±	II	±	II
7	Good	+	III	+	—	—	..	..	III
8	Good	+	—	—	—	—	..	..	IV
9	Good	+	II	+	II	+	..	..	II
10	Slimy	—	—	—	..	..	..	..	II sub-group
11	Fair	±	I	±	I	×	I	×	I
12	Salivary	—	—	—	—	—	—	—	IV
13	Good	+	II	+	II	±	II	+	II
14	Good	+	I	±	I	×	..	..	I
15	Salivary	—	—	—	—	—	I	+	I
16	Good	+	I	+	I	+	I	+	I
17	Good	+	II	+	..	..	II	+	II
18	Fair	+	III	+	..	..	—	—	III
19	Good	+	—	—	..	..	—	—	IV
20	Good	+	I	+	..	..	I	+	I
21	Salivary	—	—	—	..	..	..	..	I
22	Good	+	—	—	—	—	—	—	IV
23	Salivary	—	—	—	..	..	—	—	Streptococcus
24	Mucoid	—	—	—	—	—	—	—	Streptococcus
25	Good	+	III	+	III	+	..	..	III

* The symbols indicate, for degree of reaction: +, marked reaction; ±, fair reaction; ×, slight reaction; for type: —, no reaction; .., test not done.

and the greater cleanliness. It would seem that the rapid method, checked by the Avery method, would suffice for the majority of specimens if they are satisfactory in quality. The only necessity for mouse inoculation would be when the specimen is obviously unsatisfactory for the foregoing methods or when neither of these methods gives a reaction with type serums. Even without mice, which are difficult often to obtain, the two methods could be employed with considerable success as a preliminary to serum administration. With satisfactory specimens, few cases would fail to receive the serum because of unsuccessful tests. For statistical purposes as to incidence of type, the methods would naturally give somewhat incorrect results.

The advantages of the rapid method, in that one can supply the messenger delivering the sputum with the appropriate serum in most of the cases for which type serums are available, and the great gain to the patient through earlier administration of the serum need little comment. These advantages should help materially in popularizing the administration of serum to patients outside of the larger hospitals.

Addenda—Since the publication of the above data, a total of 183 sputa have been examined by this method with the results given in the following table. The quality of the specimens is noted and the percentages show very clearly the necessity of good specimens if successful results are to be obtained.

RESULTS—183 SPUTA, ACCORDING TO TYPE (GROUP) PRESENT.

Quality of Sputa.	Type I.			Type II.*			Type III.			Group IV.
	Number.	Number Positive.	Per Cent. Positive.	Number.	Number Positive.	Per Cent. Positive.	Number.	Number Positive.	Per Cent. Positive.	Number.
Good	40	39	97%	34	33	97%	25	23	92%	30
Poor	10	0	0%	5	0	0%	1	0	0%	38
Total	50	39	78%	39	33	84%	26	23	88%	68

* Sub-group, 4 specimens, not included in table, 2 good, of which 1 gave very faint reaction.

A RAPID METHOD FOR THE PRODUCTION OF PRECIPITIN ANTIGEN FROM BACTERIA: AN ATTEMPT TO APPLY IT TO THE DETERMINATION OF THE TYPE OF PNEUMOCOCCUS IN SPUTUM.*

Charles Krumwiede, Jr., and W. Carey Noble.

Various methods have been devised for obtaining a precipitin antigen from bacteria. Many of these require prolonged incubation of broth cultures or more or less complicated methods of extraction where growth on solid media is used. In some attempts to use the precipitin reaction to determine the presence of antigen in sputa and feces, we developed the following simple and rapid method of making a bacterial precipitin antigen which seems applicable to most and probably to all bacteria with the exception of the acid fast types.

To a heavy suspension of bacteria in distilled water, add sufficient alkaline hypochlorite solution[1] to give a final concentration of 5 per cent.; boil over the free flame or heat in a water bath for several minutes. If the bacteria have not dissolved, add more of the alkaline hypochlorite solution and reheat. Repeat this process till the bacteria are completely dissolved. This is shown by the appearance of translucence. Add several drops of an alcoholic solution of phenolphthalein. Then add $\frac{N}{1}$ hydrochloric acid till the color is just discharged. To this neutralized solution, add several volumes of 95 per cent. alcohol. A copious precipitate which increases on standing, should result. The precipitate is collected by centrifuging and decanting the supernatant fluid. Add normal saline solution to the sediment to give approximately a one to twenty solution by volume and extract at the temperature of boiling water or boil over the free flame for from three to five minutes. Then centrifuge to clear the solution of the insoluble debris. The supernatant fluid is the finished antigen. It can be prepared in a half hour or less if necessary. This antigen can be reheated for sterilization at 100° C., apparently indefinitely.

Certain points must be considered in carrying out the various steps of the process if a concentrated and economical antigen is to be obtained. Although broth cultures concentrated by sedimentation in the centrifuge may be used with success, it is preferable, wherever possible, to use distilled water suspensions of organisms grown over large areas of agar.

The initial suspension of organisms must be relatively heavy, in fact, as little fluid as is needed should be used in making the suspension. Furthermore, it must not be diluted unduly in adding the alkaline hypochlorite solution or in the process of neutralization. The alkaline hypochlorite solution should be added as concentrated as is feasible in measuring the desired amounts. If the neutralized extract is very concentrated, the addition of several volumes of alcohol will give a prompt, copious and almost complete precipitation or so nearly complete that it would not be economical to try to precipitate the remainder by further addition of alcohol. If, however, the extract is dilute, proportionately more alcohol is needed to obtain a precipitate and this separates more slowly. Even in extracts only mod-

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* Published in the Journal of Immunology, 1918, 3, p. 1.

[1] For convenience we have used the solution marketed under the trade name of "antiformin."

erately dilute the addition of more than several volumes of alcohol may only cloud the solution and flocculation will be very slight and much delayed.

The finished antigen, even after centrifuging, may be slightly opalescent. This is due to the presence of fine particles of bacterial debris, which are difficult to throw down. As the concentrated antigen may be diluted from ten to forty times or more and as the diluted antigen is very clear this opalescence is not a factor. If for some special purpose it is necessary to use the antigen in its concentrated form, the presence of this bacterial debris must be considered as it is agglutinable and will therefore bind antibody and constitute part of the precipitate. In one instance we collected the insoluble debris after boiling typhoid bacilli in a 30 per cent. solution of "antiformin" for one-half hour; we washed the debris till no precipitable substance was present in the washings and then suspended the debris in normal saline. With dilutions of serum that gave precipitation with a finished antigen, the bacterial debris was promptly agglutinated. The control suspension without serum flocculated to some extent and settled out. Even the severe treatment given did not destroy the agglutinability of the bacterial "rests." The presence of the fine debris can be avoided to some extent by not breaking up the sediment too vigorously in making the final extract. If for some special reason the debris is objectionable it can be removed by filtration.

The most striking point in the method outlined above is the extreme resistance of the precipitable substances to the prolonged heating in alkaline hypochlorite solutions, which allows the rapid solution of dense suspensions with subsequent concentration of antigen. This is really the only original step in the method as extraction with cold alkaline hypochlorite solution has been used before. The other steps are all well known. That which is new in the method is the combination of these steps so as to allow the rapid preparation of antigen freed from extraneous substances which have been added in the process of preparation. We have prepared antigens by this method from pneumococci using the sediment from centrifugalized broth cultures; from agar cultures of *B. typhosus,* types of *B. paratyphosis, B. diphtheria* and *B. mallei* and have obtained such satisfactory results from all that we see no reason why the method is not applicable to all bacteria soluble in an alkaline hypochlorite solution.

Two questions arise in relation to this method. In the process how much precipitable substance is destroyed? We attempted to determine this by preparing antigens with different concentrations of alkaline hypochlorite solutions and employing different periods of heating. By preparing these so that the concentration of the end product was as comparable as possible we could see no evidence that there was any appreciable destruction of antigen.

The other point is how far specificity is injured if at all, by the severe treatment. If the factors entering the immune reaction are properly adjusted there is no evidence of loss of specificity. For this result there must be: standardization of antigen determined by dilution and the addition of varied dilutions of immune serum, with the subsequent employment of graded dilutions of the serum against the standard of antigen thus determined, as well as due consideration of the time factor; viz., the rapidity of the reaction. If any of these factors are overlooked, especially if the antigen is too concentrated, cross reactions become numerous and marked, especially with closely allied organisms.

TABLE 1.

PRECIPITIN REACTIONS—PNEUMOCOCCUS SERA.

PNEUMOCCUS ANTIGEN.	TIME, HOURS.	SERUM TYPES AND DILUTIONS.								
		Type I.			Type II.			Type III.		
		1–1.	1–4.	1–9.	1–1.	1–4.	1–9.	1–1.	1–4.	1–9.
Type I............	1	++	+	±	—	—	—	—	—	—
	2	+++	++	+|	±	—	—	—	—	—
Type II............	1	—	—	—	++	++	+	—	—	—
	2	±	—	—	+++	+++	++	—	—	—
Type III............	1	—	—	—	—	—	—	+	—	—
	2	—	—	—	—	—	—	+!	±	—

Antigen used = 0.2 c.c. Serum solution = 0.2 c.c
SYMBOLS: +++ = profuse precipitate; ++, +|, +, ±, —, = decreasing amounts of precipitate.

With types of bacteria that can be easily separated because of their distinctly different agglutinability even a relatively concentrated antigen gives little evidence of cross reaction; that is, the antigen so prepared loses none of its specificity. The following table illustrates this, only a slight cross being noted after long incubation. The antigens were made from broth cultures treated with antiformin and prepared according to the method already given above.

This is not the place to go into the question of the relative specificity of precipitins and of agglutinins. Because we encountered more marked cross reactions with the precipitin reactions with very closly allied bacteria than was evident using the same serum for agglutination reactions, we feared that we were destroying some specificity in these cases. To exclude this possibility we prepared antigens according to the accepted method, that is, by prolonged growth in broth and subsequent removal of bacteria by filtration and compared the results obtained from the use of such antigens with those given by antigens prepared according to our method. With due regard to the factors mentioned above, that is, appropriate dilutions of antigens and of sera and consideration of the time factor, both types of antigens gave comparable results. The broth antigens being very much more dilute, did not introduce the factor of concentration of the antigens so markedly. The following tables are representative of the results obtained.

No final method of standardizing the antigens has been evolved. A relatively satisfactory method, however, is to determine the volume of sediment obtained by alcoholic precipitation, using graduated centrifuge tubes, and making the final suspension by adding saline up to twenty volumes. In this way with comparable antigens, after having obtained the appropriate dilution of one antigen against its homologous serum other antigens can be similarly diluted in testing cross reactions of this serum. This work was not carried further because the practical application of the precipitin reaction for differentiation of bacteria is relatively little used. The main interest of the method, in our mind is its great value in preparing material for teaching and demonstration purposes and for courses in immunology. It should also be of value in experimental procedures where a readily obtainable supply of concentrated antigen is needed.

We have attempted to apply the method for diagnostic purposes in the extraction of antigen from feces and from sputa. The object in attempting to extract antigen from feces was to determine whether we could detect typhoid antigen in stools and in this way determine the presence of typhoid or allied bacilli without the necessity of a prolonged bacteriological examination. Thus far such attempts have been without success. Even extracts of feces in which 95 per cent. of the bacterial flora are typhoid bacilli fail to yield a precipitable extract. Evidently we are extracting other substances from the feces which appear in the final antigens and interfere with the reaction. Further work is in progress to determine whether we can eliminate the inhibiting factors and to determine their character.

The application of this method in preparing antigens from sputa was attempted with the hope of evolving a rapid method of determining the type of pneumococcus present. Modifications have been found necessary

TABLE 2.

COMPARISON OF BROTH ANTIGENS AND ANTIGENS BY ANTIFORMIN METHOD

Organism.	Serum Dilution (Anti-typhoid)	Broth Antigen (Undiluted).			Antiformin Antigen Diluted 1:10 (Antigen A)			Antiformin Antigen Diluted 1:80† (Antigen A).		
		One-half Hour	One Hour.	Ice Box.*	One-half Hour	One Hour.	Ice Box.	One-half Hour	One Hour.	Ice Box.
B. typhosus	1:1	++	++	++	++	++	++	+	+	+
	1:6	+|	+|	+|	++	++	++	+	+	+
	1:12	+|	+|	+|	+	+|	+|	+	+	+
B. paratyph "A"	1:1	C	±	±	C	±	+	—	—	—
	1:6	—	—	±	—	±	+	—	—	—
	1:12	—	—	±	—	±	+	—	—	—
B. paratyph "B"	1:1	—	—	±	±	±	+	—	±	±
	1:6	—	—	±	—	Sl.C	±	—	—	±
	1:12	—	—	—	—	—	±	—	—	±
B. pullorum	1:1	+	++	++	+	+|	+|	—	±	±
	1:6	+	+|	+|	+	+	+|	—	±	±
	1:12	+	+	+	±	+	+|	—	±	±
B. sanguinarium	1:1	++	++	++	+|	+|	++	+	+	+
	1:6	+|	+|	+|	+|	+|	+|	+	+	+
	1:12	+	+	+|	±	+	+|	+	+	+
B. abortus equi	1:1	—	Sl.C	±	Sl.C	±	±	—	—	—
	1:6	—	—	—	Sl.C	±	±	—	—	—
	1:12	—	—	—	Sl.C	±	±	—	—	—
B. coli	1:1	—	—	—	—	—	±	—	—	—
	1:6	—	—	—	—	—	±	—	—	—
	1:12	—	—	—	—	—	±	—	—	—

Sl.C = slightly cloudy; C = cloudy; ± = slight flocculation; + = distinct flocculation; +| flocculation more marked ++ = heavy precipitate. Antigen 0.2 c.c. and serum dilution 0.2 c.c. used.

* Overnight.

† Symbols in these columns not comparable with those of very dilute antigen in Table 3. The symbols in each table are in comparison with most voluminous precipitate obtained in series representated by a table.

and although the method used at present is far from satisfactory and has given only positive results in a relatively small percentage of the specimens examined, it is presented in the hope that it will serve as a basis to which further improvements can be added. We have been handicapped by lack

TABLE 3.

COMPARISON OF BROTH ANTIGENS AND ANTIGENS BY ANTIFORMIN METHOD.

Organism.	Serum Dilution (Anti-typhoid).	Broth Antigen (1:5)			Antiformin Antigen (1:100) (Antigen B).		
		One-half Hour.	One Hour.	Ice Box.†	One-half Hour.	One Hour.	Ice Box.
B. typhosus	1:10	+|	++	++	+|	++	++
	1:20	+|	+|	++	+|	++	++
	1:50	+	+|	+|	±	+|	+|
	1:75	C	+	+	—	±	+|
	1:100	—	±	+	—	C	+
B. paratyph "A"	1:10	—	Sl.C	Sl.C	—	±	+|
	1:20	—	—	—	—	Sl.C	+
	1:50	—	—	—	—	—	+
	1:75	—	—	—	—	—	—
	1:100	—	—	—	—	—	—
B. paratyph "B"	1:10	C	C	±	—	±	+|
	1:20	—	—	—	—	Sl.C	+
	1:50	—	—	—	—	—	—
	1:75	—	—	—	—	—	—
	1:100	—	—	—	—	—	—
B. pullorum*	1:10	+|	++	++	++	++	++
	1:20	+|	+|	++	+|	++	++
	1:50	±	+	+	±	+|	+|
	1:75	Sl.C	±	+	—	+	+|
	1:100	—	C	+	—	C	+
B. sanguinarium*	1:10	+|	++	++	++	++	++
	1:20	+|	+|	++	+|	++	++
	1:50	+	+|	+|	C	+|	+|
	1:75	C	C	+	—	+	+|
	1:100	—	—	+	—	C	+
B. Abortus equinus	1:10	C	C	C	—	—	±
	1:20	—	—	—	—	—	±
	1:50	—	—	—	—	—	—
	1:75	—	—	—	—	—	—
	1:100	—	—	—	—	—	—
B. coli	1:10	C	C	±	—	—	—
	1:20	—	—	—	—	—	—
	1:50	—	—	—	—	—	—
	1:75	—	—	—	—	—	—
	1:100	—	—	—	—	—	—

Sl.C = slightly cloudy; C = cloudy; ± = slight flocculation; + = distinct flocculation; +| = more marked flocculation; ++ = heavy precipitate. Antigen — 0.2 c.c. and serum dilution 0.2 c.c. used.

* These two paratyphoid types of fowl origin were selected because of their very close agglutinative relationship one to the other and to *B. typhosus*. The extreme cross precipitation with these types in this series is no indication of loss of specificity. Compare with similar agglutinative results of Smith and Ten Broeck. (Jour. Med. Research, 1915, xxxi, 549.)

† Overnight.

of satisfactory material and further work will not be possible till the next pneumonia season.[2]

The success of the method as applied to sputum depends on several factors. One difficulty is that not infrequently the sputum submitted is salivary or pharyneal and not a specimen raised by coughing. Naturally a positive result can be obtained only if the sputum is comparatively rich in pneumococci. The technical difficulties are first, the necessity of removing the albuminous and mucous material which otherwise passes over to the final extract, making it thick or gelatinous; secondly, the fact that the alcohol will not give a sufficiently prompt and complete precipitation where little antigen is present. The method has been modified in the attempt to overcome the first difficulty mentioned.

To the sputum add sufficient antiformin to give a concentration of from 3 to 5 per cent. If the sputum is thin, add the concentrated reagent; if it is very thick, dilute by adding one-half its volume of the appropriately diluted antiformin; that is, no more dilution should be made than is necessary for final digestion. The mixture is then heated to 100° C. and shaken till it becomes fluid. Add a few drops of phenolphthalein to the hot antiformin-sputum and neutralize with acid. Then add a sufficient excess of acid to cause coagulation of the coagulable substances in the fluid, centrifugalize and collect the supernatent fluid; neutralize and add several volumes of alcohol, making a sufficient mixture to fill a 15 c.c. test tube. If a precipitate does not separate, let the tubes stand for awhile; then if a precipitate is formed, centrifugalize and decant the supernatant fluid; add 1 cc. of saline and heat to extract the sediment and centrifuge to clear. (If too much saline is added the extract may be too dilute and beyond the reaction stage.) The supernatant fluid is then added to the tubes containing the "type" sera and the tubes are incubated in the water bath. Each tube contains 0.2 c.c. of serum and 0.2 c.c of antigenic fluid. The rapidity of the reaction varies with the antigenic content of this fluid. With a fluid that is rich in antigen, there may be seen an immediate clouding followed by a prompt separation of the precipitate, if serum and antigen are mixed in the tube, or by a heavy ring of precipitate if the antigen is carefully "layered" on the serum. With a fluid less rich in antigen, the reaction is slower; with little antigen, it may be absent. Should the finished antigen be thick an attempt may be made to use it after dilution, but this is usually unsuccessful.

As is evident the method as evolved thus far is not wholly satisfactory, but even in its present state, sputa rich in antigen can be examined and the type of pneumococcus diagnosed in from one-half to one hour. Any increase in the number of positive reactions and in the rapidity of the reaction will depend on overcoming the technical difficulties already mentioned. Thus far no false reactions have been encountered unless the incubation was unduly prolonged. These may be found to occur occasionally because of the presence of much extraneous material in the finished antigen.

One fact has impressed us very strongly in carrying out the above work; viz., the very marked difference between bacterial and serum precipitation in regard to the dilution limits of the antigen. A serum antigen—as is well known—may be diluted as much as 1 to 50,000 times and still give a precipitate with a highly potent homologous immune serum. A bacterial antigen must be much more concentrated. The alcoholic precipitate, as described above when dissolved in twenty times its volume of saline cannot be diluted more than eighty or one hundred times at most. It may be that this is due to the fact that only a small fraction of the bacterial protein is precipitable. Another difference is the extraordinary resistance of the bacterial antigen to the action of heat and of chemical agents. Acidification and boiling are without effect. In fact we have cleared antigens by adding albumin then coagulating it by heat and by acidification and neutralizing the filtrate.

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[2] Three successive Type 1 sputa just received have been positive.

Summary

A simple method of preparing a precipitin antigen from bacteria is presented, which allows of the preparation of such antigens with a rapidity and in a concentration hitherto impossible. An attempt to modify the method to extract antigen from pneumonic sputum to determine rapidly the type of pneumococcus present has been partially successful. It is presented as a possible basis for further improvements.

THE POSSIBLE APPLICATION OF THE MACROSCOPIC SLIDE AGGLUTINATION IN THE SEARCH FOR MENINGOCOCCUS CARRIERS.*

Charles Krumwiede.

The methods generally employed for the detection of meningococcus carriers, namely—the streaking of plates to obtain disrete colonies, the examination of these cultures in smear stained with the Gram stain and the determination of their agglutinative properties in one or more serums, as well as the making of further subcultures necessary to determine the fermentative reaction of suspected strains, make the examination of a large number of suspects extremely laborious. The removal of the necessity for any of these steps would result in a great saving of time, labor and materials.

The use of the macroscopic slide agglutination has proven to be a time saving procedure in the methods we employ for the identification of suspected typhoid-paratyphoid colonies from feces. From this fact we were led to try this method for the meningococcus. Meningococcus organisms obtained from colonies or even from traces of growth on slant agar media show a prompt agglutination with the curative serums obtained from the horse. These serums have been found by previous test to have a high content of agglutinins, that is, a high titer and a broad range of action on various representative strains.

Two such immune serums have been tested in a 1: 10 dilution with normal horse serum (1: 10) as a control. Both immune serums gave immediate agglutination with over sixty strains representative of the great majority of strains likely to be encountered. In a few instances out of the total number of cultures, slight clumping was present in the control normal serum; but this was not due to the serum as the same result was noted with saline solution.

Eight other normal horse serums showed no action on six representative strains when used in a dilution of 1: 10. In those instances in which slight clumping occurred in the control, the immune serum result was so marked as to leave no question as to the specific agglutination of the meningococci. Higher dilutions were employed and fairly good results were obtained with a dilution of 1:25, although with some strains the size of the clumps was lessened and some delay in the reaction resulted. A 1:50 dilution gave in general unsatisfactory results. Naturally the dilution applicable would have to be determined for each serum by testing it against a series of representative strains.

The method is to place a row of small drops of the dilution of normal horse serum and another row of drops of the dilution of immune serum, on a slide. The growth from the suspected colony or a trace from a slant medium is then taken up with a small loop and rubbed first in the normal and then in the immune serum without burning the loop between, till sufficient growth is rubbed off to give a slight turbidity. The reaction in the immune serum is evidenced by the immediate or prompt development of plain visible clumps.

The applicability of the slide reaction as well as its value as a final identification method can be determined only in actual practice.[1] Several factors will have to be determined: first, possible meningococcus strains not influenced by the serum used; second, the possibility of spontaneous agglutination among strains fresh from the human body; third, false positive reactions due to the action of normal or group agglutinins on bacteria other than meningococci.

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* Published in Jour. Am. Med. Assoc., 1917, 69, p. 358.

[1] Williams, W. A. Meningococcus Carriers, page 324.

If a check is found to be necessary to exclude false reactions due to other bacteria, this may be obtained by the examination of smears. In these, meningococci would appear as typical gram negative cocci, showing the characteristic irregularity in size and intensity of staining ("autolysis").

The serum drops with culture added are allowed to dry and have served, in our experience, as satisfactory smears for the Gram stain, thus avoiding the necessity of making extra smears for morphologic examination.

NINHYDRIN REACTION IN THE EXAMINATION OF CEREBROSPINAL FLUID.*

N. Novick.

In 1915 Noble[1] described the ninhydrin reaction, a chemical test which he used in the examination of spinal fluids. He asserted that it was of value in differentiating tuberculous meningitis from other meningeal infections yielding a clear fluid.

It is well known that in tuberculous meningitis and in poliomyelitis the cerebrospinal fluid is almost invariably clear except for a slight haziness. This haziness occurs frequently and is seen by transmitted light. The cytology of the two diseases is similar, presenting a predominance of mononuclear cells ranging from 50 to nearly 100 per cent. The albumin and globulin content is generally higher in tuberculous meningitis and lower in poliomyelitis. Deviations are met with, and, therefore, differentiation by examination of spinal fluid is not easy. In doubtful cases, the tubercle bacilli may be demonstrated in the smear or by animal inoculation, but the results of the latter cannot be known for four weeks.

The ninhydrin test is simple to perform, much easier indeed than the Noguchi-globulin test. One-tenth cubic centimeter of a watery solution of 1 per cent. ninhydrin is added to 0.5 c.c. of spinal fluid in a test tube, and is then shaken and boiled for a few seconds. On cooling, a purple

TABLE 1.

EXAMINATION OF 20 CASES OF TUBERCULOUS MENINGITIS; FLUIDS CLEAR.

Case.	Albumin.	Globulin.	Ninhydrin.	Remarks.
1	++++	++++	++++	
2	++|	++|	++	Bacilli in smear.
3	++++	++++	++++	
4	++++	++++	++++	Bacilli in smear.
5	++	++	+¦	
6	+++	+++	++	
7	++++	++++	++++	Bacilli in smear.
8	+++	+++	+++	
9	+++	+++	+++	Bacilli in smear.
10	+++	+++	++	Bacilli in smear.
11	++	++	++	
12	++	++	++	
13	+++	+++	++	
14	+++	+++	++	
15	++++	++++	++++	
16	+++	+++	++	
17	+++	++|	++¦	
18	+++	+++	+++	
19	++++	++++	++++	
20	++++	++++	+++	

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* Published in Jour. Infect. Dis., 1917, 21, p. 52.

[1] München. med. Wchnschr., 1915, 62, pp. 975 and 1786.

TABLE 2.

EXAMINATION OF 15 CASES OF MENINGISM; FLUIDS CLEAR.

Case.	Albumin.	Globulin.	Ninhydrin.	Remarks.
1	+	+	+	Pneumonia.
2	+	+	+	Diphtheria.
3	—	+	+	Pneumonia.
4	+	+	+	Pneumonia.
5	—	—	—	Adenitis.
6	+	+	+	Peritonitis.
7	+	+	+	Gastro-enteritis.
8	+	+	+	Endocarditis.
9	+	+	+	Pneumonia.
10	+	+	+	Mastoid.
11	+	+	+	Pneumonia.
12	+	+	+	Pneumonia.
13	+	+	+	Pneumonia.
14	+	+	+	Pneumonia.
15	+	+	+	Pneumonia.

TABLE 3.

EXAMINATION OF 35 CASES OF POLIOMYELITIS; FLUIDS CLEAR.

Case	Albumin.	Globulin.	Ninhydrin.	Remarks.
1	+	+	+	
2	+|	+|	+|	
3	+|	+|	+	
4	+|	+|	+	
5	++++	++++	+++	
6	++++	++++	++	
7	+	+	+	
8	+|	+|	+	
9	+	+	+	
10	+|	+	+	
11	+|	+|	+	
12	++	++	+	
13	+	+	+	
14	++	+|	+	
15	+	++	+	
16	+	+	++	
17	+|	++	+|	
18	++++	++++	++++	
19	++	++	+	Fluid cloudy.
20	+	+	+	Fluid cloudy.
21	+++	+|	+	
22	++	++	++	
23	++	+|	+	
24	++	++	++	
25	+|	+|	+	
26	+|	+|	|	
27	+++	+++	++	
28	+	+	+	
29	++	++	++	
30	++	++	+	
31	+|	++	+	
32	+	+	+	
33	+	+	+	
34	+	+	+	
35	++	++	++	

TABLE 4.

EXAMINATION OF 29 CASES OF NONTUBERCULOUS MENINGITIS; FLUIDS TURBID.

Case.	Albumin.	Globulin.	Ninhydrin.	Remarks.
1	++++	++++	++++	Epidemic meningitis.
2	++	++|	++	Epidemic meningitis.
3	++++	++++	+++	Epidemic meningitis.
4	+++	+++	++	Epidemic meningitis.
5	+++	+++	+++	Epidemic meningitis.
6	+++	+++	++	Epidemic meningitis.
7	++	++	++	Epidemic meningitis.
8	++	++	++	Epidemic meningitis.
9	+++	+++	+++	Epidemic meningitis.
10	+++	+++	+++	Epidemic meningitis.
11	+++	+++	+++	Influenzal meningitis.
12	++++	++++	++++	Influenzal meningitis.
13	+++	+++	+++	Staphylococcus meningitis.
14	++++	++++	++++	Pneumococcus meningitis.
15	++	++	++	Epidemic meningitis.
16	++	++	++	Epidemic meningitis.
17	++	++	++	Epidemic meningitis.
18	++++	++++	++++	Pneumococcus meningitis.
19	++++	++++	++++	Pneumococcus meningitis.
20	++++	++++	++++	Epidemic meningitis.
21	++++	++++	++++	Epidemic meningitis.
22	++++	++++	++++	Epidemic meningitis.
23	++	++	++	Epidemic meningitis.
24	+++	+++	++++	Epidemic meningitis.
25	++++	++++	++++	Epidemic meningitis.
26	++++	++++	++++	Epidemic meningitis.
27	++++	++++	++++	Epidemic meningitis.
28	++	+++	++	Epidemic meningitis.
29	++++	++++	++++	Pneumococcus meningitis.

color turning into a deep blue appears. This indicates a positive reaction. A negative reaction gives either a straw-yellow color or no color at all. Intermediate shades of a plum color are noted, indicating a weakly positive reaction. Translated in terms of + signs, which are used in the examination of albumin* and globulin,* the colors of the ninhydrin reaction would correspond thus: Deep-blue = ++++; straw-yellow or colorless = —; plum-color = +| or ++.

Tables 1-4 give the results of examinations of the spinal fluids in 20 cases of tuberculous meningitis, 15 cases of meningism, 29 cases of nontuberculous meningitis, and 35 cases of epidemic poliomyelitis.

A study of the tables shows that a distinct relationship exists between the albumin and globulin content and the ninhydrin reaction in any given fluid. There are 20 cases of tuberculous meningitis, in 5 of which tubercle bacilli were demonstrated in the smear. The ninhydrin reaction showed no greater intensity of color than the albumin and globulin would indicate. In the examination of the cerebrospinal fluids in 15 cases of meningism, in which there were only traces of albumin and globulin, the ninhydrin gave a corresponding faint reaction. In the 35 cases of poliomyelitis, the albumin and globulin content in the fluids ran, in general, parallel with the ninhydrin reaction. The relationship between the quantity of albumin and globulin and the intensity of the ninhydrin reaction was shown again by the results obtained in the examination of the fluids in 29 cases of meningitis. In these fluids also the globulin content ran generally parallel with the ninhydrin reaction.

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* A small amount of globulin or albumin is represented by ±; increasing amounts +, ++, +++, ++++.

CONCLUSIONS

The ninhydrin reaction in the examination of spinal fluids is of moderate differential diagnostic value.

It runs almost parallel with the albumin and globulin content of a given fluid.

It may be used as a bedside presumptive test, but has no advantage over the albumin and globulin test, and is incomplete without the cytologic and bacteriologic examination, as it does not separate the nonpurulent pathologic fluids.

MENINGOCOCCUS CARRIERS.

ANNA W. WILLIAMS, GEORGIA M. COOPER, H. D. HUSSEY, A. G. MANN.

That people who have been in recent contact with cases of epidemic cerebrospinal meningitis may harbor in their throats the microörganism (meningococcus) causing this disease, has been known for some years. These people do not contract the disease themselves if they are not susceptible, but they may pass the organism on to others who are susceptible and who may therefore have a fatal attack of meningitis.

During the epidemic of 1905-6, two workers in our laboratory, Goodwin and Sholly, corroborating the work of others found meningococci in most of the throats of cases suffering from this disease, in 12 per cent. of the throats of those in recent contact with cases and in 4 per cent. of those who had not been known to be in contact with cases. Since then different observers have obtained similar results with percentages varying slightly according to the method used for identification, according to the time of the year and according to the length and closeness of contact. Carriers of the meningococcus have been found to retain the organisms in their throats for varying lengths of time. Some, about half of the number, get rid of the germs very quickly, that is, in about two weeks; about four-tenths of them carry the organisms for an average of a month and about a tenth of the number retain the organism for a much longer time. Some of the last group may carry the organisms indefinitely in spite of any treatment that may be used to try to free them from these invaders.

Since the recent war, many investigations have been carried on in regard to the prevalence of and danger from carriers of the meningococcus. The dread that "camp fever" (as cerebro-spinal meningitis has been called when it has swept through military camps) might suddenly become widely prevalent in the army and navy, gave impetus to the endeavors to discover the number of carriers, the actual danger from them and the best way of treating them.

The first question raised was: Are the tests used sufficient to prove that the strains found in the carriers are exactly similar to the strains found in the cases of cerebro-spinal meningitis? That is, are they all true pathogenic meningococci, or may not some of them be simply morphologically and culturally similar and yet be not at all pathogenic? Such a condition has been found with other groups of organisms closely studied, for example, with the diphtheria group, the pneumococcus group and so on. In these cases, an animal test can be used to show pathogenicity and a serologic test to show specificity. With the meningococcus, an animal test cannot be satisfactorily used because thus far no animal has been shown to be regularly susceptible to inoculation with this organism. Neither have the serologic tests, until recently, promised much help because of the irregular results obtained. The agglutination test, the opsonic index test, the complement fixation test, the bacteriolytic test have all been tried, abandoned and restudied. From the most recent work of English investigators it seems possible that the agglutination test using the method of *absorbing agglutinins* may give us some aid in determining the specific relationship between carrier strains and case strains of the meningococcus. These investigators have shown that tests of the absorption of specific agglutinins from specific serums give much more regular and exact results with meningococci than do direct agglutination tests. Certain of the investigators claim that the test shows that not only are there pathogenic and non-pathogenic meningococci, but that the pathogenic meningococci are divided into four groups or types just as are the pathogenic pneumococci. That the deduction made from these investigations is not as clear cut as stated by this group of investigators, is shown by the fact that another group of English workers make another claim. They say that while the absorption method shows two irregular groups of meningococci, it does not show a marked differentiation into a pathogenic and a non-pathogenic group.

The members of the first group of workers base their deduction that there are a number of non-pathogenic meningococci among the carriers, upon their claim that all strains of meningococcus obtained from meningitis cases fall into four groups, while many of the strains from carriers do not fall into those groups. In order to work out the specific relationship of strains to each other, the serum from each strain should be absorbed by not only the producing strain but by every other strain, that is, there should be cross absorption. After absorption each serum should then be tested for its agglutinative properties by the producing strain and by each strain used for absorption. This is such a long and complicated task that few workers so far have been able to give the time required to work out enough strains to give us the limits of variation. Not only is it of great importance in connection with the carrier problem to determine the question of grouping among the meningococci, but, from the standpoint of standardization of meningococcus serum, the solving of this question is of equal importance. One dovetails into the other.

Our laboratory is now running through our case strains—about 200—in order to determine their specific agglutinin reactions by absorption. The relationship of case strains to carrier strains cannot be definitely settled until this part of the problem is solved. We have already done a little work on it from the carrier strain standpoint. The work was undertaken because of the request for our aid in the hunt for carriers in certain camps, aviation fields and ship stations in the vicinity of New York. The work has been limited somewhat by the distance of our laboratory from these places and by our inability to determine accurately the extent of contact. Nevertheless, we have two groups of strains which we are studying in connection with the case strains in each group. We think these may help in solving the important question of the relationship of carrier strain to case strain.

One of these groups came from an aviation field. The case developed in one of three car loads of soldiers coming from the south. The man had had slight symptoms for a day before arriving and the soldiers from all of the cars had mingled freely at several stops on the way up. The immediate contacts of this case were 31, and among these were 8 carriers or 25.5 per cent. In the rest of the squadron numbering 187, 23 carriers—12.3 per cent. were found. The whole number examined—218—gave 14.2 per cent. carriers.

The second group came from a receiving ship station. The immediate contacts of this case gave 24 per cent. carriers and the others gave 19 per cent. The whole number examined, 293, gave 21.5 per cent. carriers. Note difference between the percentages of the whole number of carriers of the two groups. The ship people have consistently had a higher percentage of carriers on each of the several times we have examined them. This is probably due to the fact that they were usually in more confined quarters than were groups from the aero-field and camps.

The method of procedure, always of primary consideration, is of special importance in examining large groups of people such as occur in camps because of the necessity of quickness combined with accuracy.

Our attention was first directed to the culture media that were being recommended. Meningococci are notoriously capricious in their growth, especially when first isolated from a case; consequently we need especially to study and test media to try to devise better ones. We determined that we must test our media not by seeding the plates of media with stock cultures (cultures that have been isolated for some time and frequently transplanted) but by using cultures just isolated. These were used in 1 to 50 dilutions in order to make the conditions approach more nearly those met with in the primary isolation culture. The difference in results obtained by testing in this way from those obtained with stock cultures, is marked. Among the many culture media we have tested, we have found that the so-called vitamin medium recommended by the Lloyds in England and by Huntoon in this country, gives on the average somewhat better results than any other. *With recently isolated strains this is true only when a little whole blood is added to the medium.*

The next important point in our method of detection is the use of the macroscopic slide agglutination recommended by Krumwiede for a tentative diagnosis. This shortens by twenty-four hours the time for making the diagnosis, markedly lessens the amount of apparatus used and the complexity of the test.

The method, as we now employ it, is as follows:—

1. The cultures are taken from the naso-pharynx (the point of election for the meningococcus) by rubbing over it a sterile cotton swab of pliable wire protected by a West tube (a glass tube curved at one end in order to reach the naso-pharynx more easily).

2. The tip of the swab is then rubbed over a small area on one side of a vitamin-blood-agar plate. From this area many streaks over the medium are made by a *delicate platinum* needle. The plate is then placed in the incubator at 37 degrees C.

3. In about 20 hours, or the next morning, the plate is examined and fishings are made from suspicious colonies into a drop of normal serum 1 in 10 dilution and then into a drop of immune serum 1 to 10 dilution, both drops on a glass slide. Each fishing is rubbed up in the drop and the reaction noted.

If there is a homogeneous emulsion in the normal serum drop and a separation into clumps in the immune serum drop and if these drops when later dried and stained show a gram negative diplococcus, the organism is called a meningococcus and pure cultures are made from the same colony for corroboration and study. By this method a practical diagnosis of meningococcus carriers may be made within 24 hours.

The problem to be solved waits until we have settled this question of the relationship of case strain to carrier strains. If we can prove that there are fixed non-pathogenic forms and that we can differentiate practically between them and the pathogenic forms, it may be of practical worth to detect, isolate and treat carriers of the pathogenic forms; but if all micro-organisms classed as meningococcus must be considered potential pathogenic forms our chief efforts should be directed probably as in the diphtheria problem, towards discovering a test for the non-immune persons and increasing their resistance to these organisms.

AGGLUTINATION TESTS WITH THE TWO SERIES OF CASES.

Aviation Field Series.—This series consisted of one strain from a meningitis case and 31 strains from carriers who were possible contacts with the case. In addition to a serum for the case strain, these strains were tested with sera for Gordon Type I, II, III, and IV, and for the case strain of the Receiving Ship Series. By agglutination and agglutinin absorption tests, the case strain was found to be similar to Gordon Type I; also, strains from 7 carriers, 22.5 per cent. proved to be of this type. Two other strains seemed to be very closely related, although not absolutely identical with these in their reactions; that is they reduced the agglutinins of the case strain serum very markedly, but did not remove them completely.

The remaining 22 strains did not fall into any of the set types. For the most part these gave slight agglutination with all the type sera; one gave no agglutination in a 1-50 dilution with any of the sera.

Receiving Ship Series.—The case strain and strains from 38 carriers who were possible contacts with the case were studied in this series. The strains were tested with sera for the four Gordon types, a serum for the case strain and a serum for the Aviation case strain.

The Receiving Ship case strain was found to be very closely related to the Gordon Type II strain although not identical with it. This was deduced from the findings that the cross agglutinations between these two strains were very marked and that, when absorption tests were carried out, the reduction of agglutinins was very considerable with each serum. When the first agglutinin absorption tests of the case serum with Gordon Type II were done, the agglutinins seemed to be completely exhausted when tested in a dilution of 1-50. This led to the supposition that the case strain belonged to a subgroup under Type II. Later, many repetitions were made, several with lighter absorption doses. In a number of instances, the exhaustion was not complete although the agglutinins were always considerably reduced. An explanation of the difficulty encountered in obtaining clear-cut results, may be found in a comparison of the relative agglutinability of the two strains. The Gordon Type II strain is much more agglutinable than the case strain, the former giving a higher titre with the case strain serum than the latter. This difference in agglutinability was not of a temporary nature for a year and a half later results were obtained similar to the first set of observations. It was finally concluded that the case strain and the Gordon Type II strain were very closely related having many agglutinogens in common, but as neither one possessed all the agglutinogens belonging to the other, neither is a dominant type and there is not a subgroup relationship between them.

Nineteen (50 per cent.) of the strains were discovered to be similar to the case strain. Tests with other Type Sera showed 4 of these strains to be Gordon Type I. The remaining 15 did not fall into any of the set types; 7 gave slight agglutination with all the type sera, 5 gave slight agglutination with Types I and III, 3 gave no agglutination with any of the sera in a dilution of 1-50.

The percentage of strains similar to the case strains, 22.5 per cent. in one series and 50 per cent. in the other, indicates the extent to which the case type had been disseminated among these contact carriers.

COMMON COLDS AS A POSSIBLE SOURCE OF CONTAGION FOR LOBAR PNEUMONIA.*

Eugenia Valentine.

The pneumococcus has been assumed to be one of the causative agents in common colds because of its known pathogenic character and because of its predominance in the mucous discharge in some cases. As far as we know, determination of the types of pneumococci encountered in these conditions has not been made except in as far as the *Pneumococcus mucosus* type was identified on cultural grounds. For this reason, a series of common colds was examined to determine the types of pneumococci present. This was undertaken not so much from the standpoint of determining the etiological significance of pneumococci in common colds, but rather to determine whether the fixed types occurred. If these were found, common colds would then have to be considered as a source of infection in the spread of lobar pneumonia.

By injecting the nasal secretion or sputum into mice, pneumococci were recovered in 37 out of 65 cases of common colds. By direct plating on blood agar, pneumococci were found in 6 additional cases. Of the total 43 cases, the incidence of type or group of pneumococcus recovered was as follows: Group I, 2 cases; Group II, 2 cases; Group III, 4 cases; Group IV, 35 cases. The cases from which Group II was isolated and one of the Type III cases had been in contact with pneumonia and, with the data available, it is impossible to decide whether they were merely contact carriers or whether the cold was an actual infection by pneumococci due to contact with the pneumonia cases. None of the other cases from which fixed types were isolated had any such contact as far as could be determined.

In determining the type, immune horse sera were employed. Agglutination to a somewhat marked extent was encountered with either or both of the horse sera (Type I and Type II) in low dilutions with the strains finally included in Group IV. In the cases where this was marked, further agglutinations were done with rabbit sera which did not give these aberrant reactions, to eliminate the possibility that some of the strains belong to the subgroups of the fixed types as described by Avery.[1] Table I gives the results of the tests and shows conclusively that the irregular reactions with the horse sera were due to non-specific agglutinins. Two of the Group IV cultures, C3 and C5, show some degree of relation in their cross agglutination.

In three of the four cases yielding Type III, mouse inoculation only was done, so that no data are available on the preponderance of this organism. In the fourth, direct cultures showed the presence of about 75 per cent. of this organism. The data available, therefore, except in one instance, are merely an indication that the catarrhal inflammation was due to this type. *Pneumococcus mucosus* has been found by other observers in the secretions of the respiratory tract in conditions other than lobar pneumonia, as well as in otherwise normal individuals. All these individuals, therefore, must be looked upon as potential sources of infection for others or as sources of possible autogenic cases of lobar pneumonia.

The recovery of pneumococci of Type I from two cases of common colds raises the question as pointed out above whether these persons were

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* Published in Jour. Exp. Med., 1918, 27, p. 27.

[1] Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

TABLE I.

Type or group.	Case Cultures.	Rabbit sera.					
		C 2.	C 3.	C 5.	C 14.	Type I.	Type II.
IV	C 1	—	—	—	—	—	—
IV	C 2	400	—	—	—	—	—
IV	C 3	—	400	100	—	—	—
IV	C 4	—	—	—	—	—	—
IV	C 5	—	100	300	—	—	—
I	C 14	—	—	—	600	800	—
I	C 20	—	—	—	600	800	—
I	Control strain	—	—	—	600	800	—
II	Control strain	—	—	—	—	—	400

carriers or whether the inflammation was due to the pneumococcus. Both patients were markedly prostrated and had a temperature of 102° F. In both cases direct plates showed that the Type I pneumococcus constituted at least 75 per cent. of the organisms developing from the secretion, which strongly suggests that the pneumococcus was the etiological agent. If this deduction is correct, we must add some cases of common cold to the already known sources of contagion for lobar pneumonia; namely, the actual case of pneumonia, the convalescent, and the contact carrier (Stillman[2]). One of these cases was reëxamined by mouse inoculation whenever a cold recurred over a period of over 2 years. The first reëxamination, done a year later, yielded a few Type I pneumococci, most of the organisms in the mouse peritoneal cavity being Pneumococcus Type IV and the bacillus of Friedländer. This shows that the individual was still a carrier of Type I, but the small numbers of this organism recovered indicate that it was not of etiological significance in this cold. Subsequent examinations for a period of a year, the first a month later, yielded only Pneumococcus Type IV.

SUMMARY.

In two instances of common colds, with no known contact with cases of pneumonia, Pneumococcus Type I was found to be the predominating organism, which strongly suggests that it was the etiological agent in these colds. If this is so, common colds of this type must be looked upon as a possible source of contagion in the development of lobar pneumonia due to the Type I pneumococcus.

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2. Stillman, E. G., *J. Exp. Med.*, 1916, xxiv, 651.

STUDIES ON THE ETIOLOGY OF POLIOMYELITIS.*

Anna W. Williams, C. R. Gurley, A. G. Mann.

Certain claims made in regard to the nature of the virus of Poliomyelitis and corroborated by many workers, must be considered by anyone bringing forth new claims as to the chief exciting cause of this disease.

These claims are briefly as follows:

1. The virus is located chiefly in the central nervous system, in and on the mucous membrane of the gastro intestinal tract, in the adjacent lymph nodes and in the salivary glands. It is *not* found to appreciable extent in the spinal fluid or in the blood.
2. The virus is filtrable, passing readily through Berkefelds, less rapidly through Chamberlands.
3. Of the many animals tried, the monkey is the only one in which a typical infection is produced by the virus.
4. The virus produces an active immunity in monkeys and the blood serum of such monkeys contains substances that neutralize the virus.

All communications that do not take these claims into consideration, either accepting them as true or showing that they are not true can be disregarded.

Only two claims at present come up to these requirements—that for the Flexner-Noguchi organism and that for the streptococcus of Rosenow.

Flexner and Noguchi[1] claim for their organism that it is found (though with difficulty) in the central nervous system of practically every monkey dying after lethal doses of poliomyelitis virus:[2]—that it has been found though less often in filtrates from the central nervous system and in washings from the gastro-intestinal tract; that it is slightly filtrable passing with difficulty through the coarser filters; finally, that it produces typical lesions in monkeys in culture generations as high as the 20th. But they have not shown that the cultures produce immunity. Their chain of evidence, therefore, as to the specificity of their cultures in etiology is incomplete.

Rosenow[3] and his co-workers claim that they have obtained a particularly irregular streptococcus from the central nervous system and the tonsils of all the human and monkey cases examined; that they found it slightly filtrable; that it produces typical lesions not only in monkeys, but in many other animals. But they have not yet shown that it produces immunity.

They state further that their streptococcus is the same organism as the minute coccoid organism obtained by Noguchi and attempt to prove this by a morphologic demonstration of the breaking off of minute forms from larger forms. They have not shown, however, that such minute forms remain minute and that they grow only anaerobically. Neither have these workers explained satisfactorily why they obtain so many more results in different animals with their cultures than with the virus.

Other workers have already claimed that the paralyses produced by streptococci in those animals susceptible to the virus (monkeys and to a less extent rabbits) are not accompanied by lesions similar to those produced by the virus.

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* Published in Proceed. N. Y. Path. Soc., 1917, 17, p. 15.

[1] Jour. Exp. Med., 1913 and 1914.

[2] No definite statements have been published as to the "finds" in human cases.

[3] Jour. Amer. Med. Assoc., 1916, 60, p. 1202.

In our work, we have followed as closely as possible the technic employed by the Rockefeller group of workers and that used by the Rosenow group as well as some methods of our own.

The autopsies were made under as aseptic precautions as possible. This work was especially satisfactory as carried out in monkeys. The brain and then the spinal cord were removed and placed in sterile receptacles. In some cases following Rosenow's method, before removal of the central nervous system, a portion of the surface was seared and a sterile capillary pipette was inserted. Some of the contents both the fluid from the ventricles and the nerve tissue from different parts of the interior of the brain and spinal cord, were removed. In other cases sharp surface cuts were often made and pieces removed with a sterile instrument.

The material was brought to the laboratory immediately and the following culture media were inoculated from different parts in most of the cases in the order given:

1. Veal agar plates with and without 0.2 per cent glucose.
2. Extract broth plus 0.2 per cent glucose.
3. Veal broth.
4. Slant egg medium with and without ascitic fluid.
5. Slant Bordet medium with and without ascitic fluid.
6. Veal horse serum agar tubes with and without 0.2 per cent glucose.
7. Ascitic kidney (prepared as recommended by Noguchi).

Most of the tubes were made with and without albolene. Some were placed in an atmosphere of nitrogen as well. From 20 to 200 initial cultures were made from each case. The cultures were put in the incubator at about 35° C. After the cultures were made, material was taken for filtering and some for inoculation of monkeys and rabbits. Other material was dropped into fixatives for sections and still other portions were put into 50 per cent glycerine and stored in the ice-box at about 7° C. for later examinations. The remaining material (not used by other workers) from many of the good autopsies was sealed and sent to a cold storage plant.

We had material from 40 human autopsies, one of which proved not to be poliomyelitis, and accordingly the material was put with the controls. Out of the thirty-nine, twenty-six showed in smears a minute streptobacterium morphologically similar to that obtained by Flexner and Noguchi; twenty-three showed irregular strepto-cocci and eighteen showed other minute organisms which are still being studied. All of the above findings were in only a few out of the many tubes inoculated for the majority of the tubes remained sterile.

It was comparatively easy to obtain pure cultures of streptococci from most of those tubes showing streptococci in the smears and pure cultures of the several minute organisms not unlike the Flexner-Noguchi organisms; but from the mixed cultures, judging morphologically, it has been impossible to obtain any anaerobic organism of the Flexner-Noguchi type. Only twice, so far, have we got pure cultures of a microorganism answering to the description of theirs and in these cases from tubes that contained originally pure growths. Fortunately, we are able to compare these organisms with those of the Rockefeller strains since Dr. Amos kindly gave us four strains of the Flexner-Noguchi germ and they have been closely studied. So far they seem quite similar.

Up to this time, we have made autopsies on fifteen monkeys inoculated with the virus and though we have found morphologically typical minute organisms mixed with medium-sized streptococci in 5 monkeys, we have not yet obtained pure cultures of the Rockefeller type of organism from these monkeys.

When we first found these minute cocci in the smears from the primary tubes mixed with other organisms, we were inclined to believe that the Rockefeller type of organism was present very frequently. However, since we have been carrying on pure successive cultures of streptococci under anaerobic conditions we have found that most strains produce some forms as minute as those of the Flexner-Noguchi type. We have, therefore, come to the conclusion that we can judge very little of the type found in mixed cultures from a morphologic study alone. Why we are not getting from the monkeys the minute organism if it is invariably present, we cannot understand. Our pure strains are growing abundantly in our ascitic fluids, showing that the fluids are suitable and we are inoculating many tubes of the material, but most tubes remain sterile.

Culture Control.—The largest numbers of our cultures were made in media to which animal tissues were added, therefore, all media had to be most carefully controlled. We found streptococci in our control tubes several times and even more frequently found a small irregular bacillus with

RESULTS FROM PRIMARY CULTURES OF FRESH MATERIAL.

Number of Cases.	Smears of Cultures.			Pure Cultures.		
	Minute Strepto-bacterium Morphologically Similar to Flexner-Noguchi.	Streptococcus.	Other Minute Organisms	Strepto-bacterium.	Streptococcus.	Other Organisms.
Human: 8......	+	+	+	..	1	4
10......	+	+	—	2	7	..
4......	+	—	+	..	..	2
4......	+	—	—	..	..	..
3......	—	+	+	..	..	1
2......	—	+	—	..	..	..
3......	—	—	+	..	..	1
5......	—	—	—	..	..	..
Monkeys: 3......	+	+	+	..	2	..
2......	+	—	+	..	..	..
4......	—	+	+	..	2	..
5......	—	+	—	..	4	..
1......	—	—	+	..	..	..

CONTROLS.

Human: 1......	—	—	—	..	1	..
Monkeys: 2......	—	+	—	..	1	..
Dogs: 1......	—	+	+	..	..	..
Cats: 1......	—	—	+	..	..	..
2......	—	—	—	..	..	..
Rabbits: 5......	—	+	—	..	2	..
1......	—	—	—	..	..	..

many minute forms growing rather slowly so early examinations did not show it. Occasionally, whole sets of our animal tissue cultures were contaminated by a small organism of this type.

Animal Experiments with Cultures.—One of our strains (P. 7) was inoculated into a monkey from the 3rd culture generation and produced in twenty-four hours a spastic paralysis in the arm opposite the lesion. In forty-eight hours the leg on the same side as the arm became stiff. Later, in two or three days, the arm became better, but the leg became weak. The leg continued weak for several weeks and is still being dragged slightly. We kept the monkey to inoculate him later with a "fixed monkey virus" to test his immunity. He proved to be immune. Of course, the third culture generations do not rule out passage of an ultramicroscopic filtrable virus. We did not have enough monkeys to test this point.

In our cultural work, so far, we have been much hampered by the lack of monkeys. For this reason we could not test our later culture generations until they were too old to promise enough to justify the use of one of our few monkeys. It has been the same with our streptococci. None of the 3 monkeys inoculated with streptococci from these cases obtained after the method of Rosenow, showed paralysis. Two rabbits out of 12 inoculated with streptococci, showed paralysis with localization of the streptococci in the central nervous system.

Conclusions.

1. A minute organism similar to the one described by Flexner and Noguchi has been isolated from the central nervous system of a very small percentage of cases of human poliomyelitis. No similar organism has been isolated from any of fifteen monkeys dying after inoculation with poliomyelitis virus.
2. No new evidence has been obtained for or against the specificity of this organism in the etiology of poliomyelitis. The organism is a species distinct from that of any aerobic streptococcus studied.
3. Streptococci of several types, those answering to Rosenow's description predominating, have been found in small numbers in the majority of these cases.

No evidence has yet been found indicating a specific etiologic relationship of any of these types to poliomyelitis.

THE ETIOLOGY OF "INFLUENZA."*

Anna W. Williams, Hazel M. Hatfield, Alice G. Mann,
Helena D. Hussey.

In planning the following investigation for the detection of a specific agent in the "Flu" pandemic, we chose those culture media and methods of obtaining material which would aid us in detecting any or all of the organisms already described as a possible cause, as far as we could judge from the meager reports, or any other organisms of signficance with the exception of a filterable virus.

We reasoned that a study of the existence of a filtrable virus to be worth anything would involve such controlled handling of human tests that it could be done best in camps with volunteers, therefore, we decided to leave it to the camp workers. So far, the few experiments made in this country for detecting a filtrable virus have been negative.[1] In France, two insufficiently controlled experiments have been reported.

We began our study of this epidemic about the middle of September. The contagious disease hospitals of New York City received groups of sailors from the Navy Yard in this vicinity so that we had the opportunity of studying a number coming down with the disease. At about the same time we obtained material, also, directly from the receiving ship in order to get some cases in the earliest possible hours of infection. In addition we made a study of some early and later cases from a camp in this vicinity.

At first we studied cultures from different parts of the naso-pharynx (nose, tonsils, entrances of posterior nares into pharynx) and from the sputum. (Secretion from the deeper air passages cannot be obtained early in the disease). We soon found that we obtained cultures less contaminated with the ordinary mouth organisms, when we took them from the pharynx at the entrance of the posterior nares. This was done by means of a curved swab protected by a West tube in the same way that cultures are obtained for the determination of the presence of meningococcus. Therefore, we took all our later cultures in early cases from this area only and called them naso-pharyngeal cultures. Our second best results were from the tonsils. Cultures from the sputum in these early cases were very unsatisfactory because many mouth organisms were present and the influenza bacilli were likely to be missed. Of course sputum obtained fresh from the deeper air passages, should contain many of the organisms especially in those cases where the infection extends downward or begins deeper than the naso-pharynx, unless it happens that the influenza bacilli cling to the mucous membrane.

For growing these cultures we chose media which we had found best for the development of influenza bacilli, of hemolytic streptococci, of pneumococci, of the streptococcus viridans group and of meningococcus-like organisms. We arranged our record cards so that we could note the presence and number of these organisms and of any other microorganisms that might be either in great abundance or have peculiar characteristics. In our hunt for unusual organisms, we grew cultures under partial anae-robiosis with negative results so far.

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* Published in Proceed. N. Y. Path. Soc., 1918, 18, p. 83.

[1] J. J. Keegan: Jour. Am. Med. Assn., 1918, lxi, 1051.

Our general method of making this study may be described as follows:

At the bedside:

1. Cultures are taken from the naso-pharynx with a swab protected by a West Tube.
2. A small area is inoculated on one side of a blood pour[2] vitamin agar plate.
3. On a blood-drop agar plate (a drop of blood placed on one side of veal agar plate) a similar small area is inoculated with the same swab.
4. The swab is then rubbed around in a tube containing blood broth (4 per cent. whole blood) and is afterwards discarded.

The two plates and the blood broth tube are carried to the laboratory.

At the laboratory:

1. With a sterile platinum loop, another blood-drop plate of vitamin agar is seeded from the inoculated area of the first by streaking out over the entire plate in many streaks from the first area.
2. The other two plates are then streaked in the same manner.
3. Two blood-pour agar plates are made by inoculating them while the blood-agar is still melted (at 45°C.) with one loop and 0.2 c.c. respectively from the blood-broth tube inoculated from the swab.

In twenty-four hours on the blood-pour agar plates, the influenza colonies appear to the naked eye as minute dew-drop colonies; on blood-drop-agar plates under the microscope, magnified 100 diameters, as small whitish areas embedded in the red blood cells. The pneumococcus and streptococcus which might be mistaken for influenza bacilli colonies, show a greenish rim; the blood cells are not so used up. The influenza colonies are most typical on this plate. They often show a characteristic "heaping" in the center. When surrounded by blood cells the margins of the colonies often appear irregularly scalloped, but on the edges of the blood mass the free margins of the colonies are usually entire with an occasional indentation. It is difficult, however, to focus on a definite marginal line as the margin seems to vanish suddenly on focusing. The blood-drop-vitamin-agar plate which is a third dilution often shows very large colonies of influenza bacilli. Transplants are made on glycerin-veal-blood agar (chocolate medium). The formula of the latter is the following:

Chocolate medium:

Veal agar set neutral to phenolphthalein and when sterilized about Ph. 7.8.
Glycerin 5 per cent.
Whole blood (horse or other), 5 per cent.
Add blood to glycerin-veal-agar just after latter has been removed from the sterilizer (at about 90° C.).

The formula for *vitamin agar* is as follows:

Beef heart, fresh, trimmed free from fat and tendon and finely chopped.
Peptone, Difco, 1.5 per cent.
Sodium chloride, 0.5 per cent.
Eggs and tap water.

Procedure A:

Beef heart	500 grams
Tap water	500 cc.
Egg well beaten	
Peptone	15 grams
Sodium Chloride	5 grams

Heat in water bath, double boiler, or over open fire with constant stirring until color changes to brown (at about 68° to 70° C.). Strain through fine wire sieve or wire gauze. Do not use cheesecloth.

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[2] Citrated horse blood was used for all of this work.

Procedure B:

Agar	15 Gm.
Tap water	500 cc.

Heat to dissolve agar, either in autoclave or over open flame. If the latter, make up the water lost by evaporation. Do this by weight. When agar is completely dissolved, cool to 70° C. and add mixture A. Set reaction 0.4 acid to phenolphthalein (room temperature titration) by adding normal sodium hydroxid, or make slightly alkaline to litmus paper. Place mixture in autoclave and heat for one-half hour at fifteen pounds pressure. Remove kettle carefully without disturbing contents and set aside to solidify. The meat particles, etc., settle gradually to the bottom. After agar hardens completely, turn it out in mould form and trim off the bottom layer containing the sediment. The remainder of the agar is fairly clear. Melt and tube or bottle. Sterilize for one-half hour at fifteen pounds pressure. Final reaction should be about Ph 7.6.

In not all cases even with the best medium do the colonies of influenza bacilli appear in twenty-four hours. Negative plates should be returned to the incubator and examined again after forty-eight and seventy-two hours before being discarded as negative.

We examined about 200 cases diagnosed clinically as influenza in from the first to the sixth day of the disease. In all of these cases we found that the earlier the case was cultured the more constantly did we find large numbers of influenza bacilli in the naso-pharynx. No other picture was so constant, though we did find a high percentage of meningococcus-like organisms of streptococci belonging to the viridans group.

We have had two groups of cases which bore out this picture strikingly.

The first group was in a Children's Home from which some nurses had been sent out to help the sufferers at a blown-up munition plant. Many cases of influenza developed among the survivors and the nurses came down with the disease just after they returned to the Home. An epidemic then swept over the institution in quite a virulent form including many more nurses and nearly all the children. We were able to culture many of the cases in the first day of the disease. These all showed abundant, almost pure cultures of influenza bacilli and 98 per cent. of the others (48 cultures in all) showed the bacilli in large numbers. Among 9 cultures taken in the Admitting Ward which was not kept strictly quarantined, 4 showed influenza bacilli; 1 from a case having a nasal discharge contained many bacilli and the others very few. Any deduction drawn from the high incidence of the influenza bacilli in the typical cases of this group was vitiated by the fact that there had been a number of cases of pertussis in the institution during the summer. In this disease the percentage of influenza bacilli is very high.

The other group consisted of eighty Marines who came from the South in a body. They all came down with the typical "Influenza" just as they arrived in New York and were sent to the hospital immediately. We cultured 30 of them and they all showed large numbers of influenza bacilli in almost pure culture in many of the cases. In this group there had been no pertussis and no measles in both of which diseases many influenza bacilli are usually found.

Our most striking control so far is from a Girl's Home containing 500 inmates where no "influenza" cases had occurred. Thirty-four cultures were taken from different groups of girls and in only 2, one of whom had a "slight cold" and had been kept isolated, were influenza bacilli found.

Since this examination was made, we have examined a Preventorium where the inmates had not been kept strictly isolated from the village in which there had been a number of cases of influenza with one typical case and the usual number of short fevers. Here we found that 33 per cent. of the cultures contained influenza bacilli.

We were able also to make a bacteriologic examination of thirty *autopsies,* the results of which are given briefly below:

In 24 cases we found influenza bacilli in the lungs, in 5 of which they were in pure culture. In 26 out of 27 tracheas in these cases, we found influenza bacilli in large numbers. We found pneumococci (mostly type IV) in 15 lungs, streptococcus hemolyticus in 9, Gram-negative cocci in 1, staphylococcus in 2 and a small Gram-negative bacillus in 1. Thus we see that the influenza bacillus was found more frequently than any other one organism, but whether or not it was the initiating cause of the disease or only a more frequent secondary invader than the other bacteria we cannot say.

All of our results in regard to the incidence of the influenza bacillus may be tabulated as follows:

B. INFLUENZAE.

	Present.	Absent.	Per Cent.
Earlier hospital cases	160	40	80.0
Marines	30	0	100.0
Home for children	47	1	98.0

CONTROLS.

	Present.	Absent.	Per Cent.
Nurses—contacts	4	6	40.0
Nurses—non-contacts	1	7	12.5
Measles	4	2	66.6
Admission ward	4	5	44.4
Home for girls	2	32	6.0
Preventorium	14	25	35.9

AUTOPSIES.

	Present.	Absent.	Per Cent.
Lungs	24	6	80.0
Pure cultures	5	25	16.6
Tracheas	26	1	96.3

Just as soon as we began to find the influenza bacilli in pure cultures in the lungs in such large numbers of early cases and in very large numbers in and between the cells in direct smears from most of the tracheas examined at autopsy, we came to the conclusion that, whether or not this bacillus is the initiating cause of the present epidemic, it is evident that it has a marked pathogenicity. This seemed to be sufficient reason for us to make a thorough trial of the effect of an influenza bacillus vaccine on the prevention, if not of the pandemic, at least of a number of the cases of infection with this bacillus.

The problem of producing immense quantities of vaccine; of determining the best dosage; of the time for immunity, if any, to be established; of getting the proper controls in communities not yet infected and above all of keeping good records and gathering and analyzing the data, was one of the most important and staggering of problems.

We began the use of our influenza vaccine after the pandemic had already started and some groups were not vaccinated until after the peak of the pandemic had been passed, so unless we could get a sufficient number of control cases, that is, presumably susceptible unvaccinated cases, we could arrive at no conclusion with regard to the efficiency of the vaccine. It is strange how difficult it has been for us to get the right kind of contacts. The reports from the use of our vaccine are being analyzed now and it is already apparent that the majority of them are lacking in many essentials.

The intelligently controlled use of the vaccine might have given us strong evidence in regard to the etiologic relationship of the influenza bacillus to the present pandemic; as it is, unless the finished reports prove to be better than they now promise, we must wait for such evidence as may be gathered from a serologic study of the various strains of the influenza bacillus isolated by us from the typical cases and from control cases in this study. We are carrying on a serologic study of influenza bacilli isolated for the purpose of gaining evidence as to the presence of a pandemic strain.

Conclusions.

The evidence in favor of the influenza bacillus being the initiating cause of the present pandemic of "influenza," is not strong enough to constitute proof.

No evidence as strong has been brought forward in favor of any other cause.

Whether or not the B. influenzae is the initiating cause, it has given evidence that it has a marked pathogenic action and the study of a vaccine to prevent this action should be continued until we are at least sure that it can give us no positive results.

ACTIVE IMMUNITY IN EXPERIMENTAL POLIOMYELITIS.*

H. L. Abramson and Herman Gerber.

The search for a method of specific inoculation against acute poliomyelitis is not a new one. Shortly after the announcement by Landsteiner and Popper[1] in 1909 of the successful transmission of poliomyelitis to members of the monkey family, workers in this country and Europe began to study the immunity problems of this disease.

Flexner and Lewis[2] in 1910 demonstrated that monkeys could be immunized against poliomyelitis by repeated subcutaneous injections with increasing amounts of a saline suspension of the crude unmodified virus. They injected animals over a period of two and one-half months. About ten days after the completion of the course of inoculation, the animals were injected intracerebrally with 2 c.c. of a filtrate of a very potent virus of which .05 to .1 c.c. would prove fatal. The animal tested presented no sign of infection, whereas the control died of poliomyelitis. In a later communication,[3] Flexner and Lewis state that they were not uniformly successful in the attempt to procure artificial active immunity either by the injection of a single large dose or by a series of increasing small doses given during a certain period of time. They found that when they gave a single large subcutaneous dose, some of the animals developed poliomyelitis as the result of this injection and after either one large or repeated small doses, some animals did not resist intracerebral inoculation of rather large doses of a highly potent virus. In the blood serum of these treated animals, the presence of neutralizing principles for the potent virus was demonstrated in good concentration.

Landsteiner and Levaditi[4] attempted to devise a method for the prevention of poliomyelitis analogous to the Pasteur method for the prevention of rabies by using cords dried for as long a period as 24 days. While some animals treated by this method developed immunity unaccompanied by any ill effects, other animals, similarly treated developed the clinical picture of experimental poliomyelitis.

Römer and Joseph[5] thought they had produced immunity in monkeys by the intracerebral injection of a mixture of virus and a serum containing neutralizing substances for the virus. They found that a monkey so inoculated resisted a subsequent intracerebral injection of straight virus sufficient to kill such an animal. However, that such a method of immunization is not invariably successful, is evidenced by the experience of Flexner and Lewis and Landsteiner and Levaditi who had no difficulty in infecting animals that previously had been injected intracerebrally with a neutralized mixture of serum and virus. Flexner and Lewis had no difficulty in infecting an animal so treated that had previously resisted an intracerebral dose of straight highly potent virus sufficient to produce death.

We can confirm these findings concerning neutralized and straight virus and further than that, we have re-infected by suitable intracerebral inoculations two animals which had been paralyzed over a year ago and which presented residual palsies at the time of the latter injection. These animals succumbed as promptly as the control animals which received a third of the dose. This experiment will be described in detail later.

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Flexner[6] stated in 1910 that while results in artificial active immunity thus far achieved were encouraging, our knowledge at that time was not sufficient to render these results of practical value. This statement has not been controverted and is the stimulus for the work which has engrossed our attention for the past year and a half.

The conditions with which we have attempted to comply in our efforts to arrive at a suitable method for the production of immunity are as follows:—

> 1. That the method shall protect against any reasonable exposure to the disease for which the individual is immunized.
> 2. That the inoculations in themselves shall be absolutely harmless.
> 3. That the method itself shall not be so cumbersome or prolonged, as to render the production of immunity too slow to be of practical value.

After an extended and rather discouraging experience with young rabbits, we turned our attention to the use of monkeys of the Rhesus variety as test animals.

The striking similarity between the characteristics of the virus of rabies and the virus of poliomyelitis suggested to us the idea of attenuating the highly potent Rockefeller poliomyelitis strain. Attempts were made to this end by two methods, one by chemical means and the other by subjecting virus to heat.

Modification of Polio Virus by Contact with .5 *per cent Formaldehyde:* —In one attempt to modify polio virus by chemical means we applied the method devised by Cummings for antirabic treatment. Cummings put a two per cent emulsion of fixed rabic virus in contact with .5 per cent Formaldehyde for four hours in the ice-box. At the end of this time, he dialyzed the formalin from the mixture through collodion sacs into distilled water until the cord emulsion failed to give test for formalin. The rabbits to be protected were then inoculated daily with this material in increasing doses.

In our experiments, we used a 10 per cent emulsion of the cords and brains of monkeys dead of highly virulent poliomyelitis virus making it up fresh for each injection. This 10 per cent emulsion was kept in contact with .5 per cent formaldehyde for four hours. It was assumed that this contact would kill the polio virus. However, our experience proved to us without any chance for doubt that the virus was not killed by this method. The protocol follows:—

Experiment. Macacus Rhesus Monkeys No. 89 and 90 were injected subcutaneously as follows:—

Mar. 28, 1917........10 c.c.
Mar. 30, 1917........15 c.c.
Apr. 2, 1917........15 c.c.
Apr. 5, 1917........15 c.c.

On April 7, 1917, No. 90 appeared ill; refused food; seemed to be tremulous; showed no sign of paralysis.

April 8, 1917, No. 90 died during the night. Postmortem showed nothing of note in abdominal or thoracic viscera. Section of cord showed some swelling and reddening of gray matter. Microscopic examination of cord showed slight perivascular cell infiltration; moderate diffuse cell infiltration; considerable nerve cell degeneration and neurophagocytosis; only slight changes in the meninges; all the classical pathologic findings of poliomyelitis.

Macacus Rhesus No. 89 remained apparently well until April 26, 1917, when it appeared to be ill. Would not feed well. Showed no paresis of any kind. Died during the night. Microscopic section of cord showed poliomyelitis.

These animals presented a rather unusual type of infection, one dying within ten days and the other within one month after the institution of the subcutaneous injections. It resembles very much the type described by Flexner as the "marantic" type of monkey poliomyelitis. In this type, the animal may be sick over a longer period of time than the animals in the above experiment, yet they do not present flaccid paralysis.

Our results with the above method were decidedly not encouraging and as monkeys were very scarce, we turned our attention to the possibility of attenuating the virus by heat.

Modification of Virus by Heat:—It has been demonstrated that poliomyelitis virus is rendered inert by exposing it to a temperature of 50° to 55° C. for one-half hour. With this fact as a basis, it was decided to attempt the immunization of animals by the use of a virus inactivated by heat. The heat was applied and the inoculations given according to two different methods. By both methods, animals were inoculated subcutaneously on successive days with a previously heated 10 per cent saline emulsion of a highly potent virus. The emulsion was made of the brains and cords of monkeys recently paralyzed by intracerebral injections of very virulent virus.

According to the first method tried, Macacus Rhesus monkeys were inoculated on four successive days with five cubic centimeters of the above emulsion each dose of which had been heated on the day of injection for one-half hour as follows:—

1st dose to 55° C.
2nd dose to 55° C.
3rd dose to 45° C.
4th dose to 37°C.

The fifth dose on the fifth day consisted of 5 c.c. of the virus emulsion unmodified by heat.

As will be seen the above method involves a graded increase in the virulence of the material inoculated, paralleling after a fashion the use of increasingly more virulent cords in the production of immunity to rabies.

By the second method tried, monkeys were inoculated on ten successive days each with 5 c.c. of the 10 per cent emulsion each dose of which had been heated to 55° C. for one-half hour. This method corresponds to the use by Semple of subcutaneous injection of killed rabic virus in the Pasteur treatment. The use of polio virus by this method does not involve the dangerous possibility of poliomyelitis in the treated animal inasmuch as the heated virus is unable to infect a monkey when injected in large amounts by the intracerebral route. In other words, the virus is apparently killed.

In testing the protective value of these two methods of vaccination we wished to determine two facts:

1. Are the animals so treated capable of resisting a multiple lethal dose of the virus intracerebrally?
2. Does the blood of animals so treated contain neutralizing principles for a highly potent polio virus?

In order to determine these points we proceeded as follows:

Three weeks after the completion of the two series of subcutaneous injections, three animals of each series were bled from the heart. The serum obtained from each of these bleedings was put into contact with a 5 per cent emulsion of highly potent Rockefeller virus in the proportion of one to one. The mixtures were kept for two hours at 37° C. followed by twenty-two hours in the ice-box. Each mixture was then inoculated intracerebrally into monkeys six cubic centimeters constituting a dose and six normal monkeys being used.

At the same time that these neutralization tests were made the treated monkeys of the first series from which the blood was drawn, were tested as to their development of immunity. Using the same emulsion of active virus employed in the neutralization tests, these monkeys were inoculated intracerebrally with from 0.15 to 0.3 c.c. of the virus emulsion.

Charts I, II and III show in detail the results given by these experiments.

Chart I shows that of five animals treated by the five injection method, three survived the test intracerebral injection of 0.3 c.c. of an emulsion of a highly potent virus fatal to monkeys in doses of .05 to 0.1 c.c. of the filtrate. The two test animals No. 91 and No. 97 that succumbed, exhibited paralysis on the sixth day after the test injection. The normal control animals No. 19 and No. 23 showed paralysis on the 4th and 5th day respectively.

There is included also in this Chart as a further control Macacus Rhesus No. 24 an animal that had survived an infection from virus of the 1916 epidemic about one year before and which at the time of the present experiment presented a residual diplegia. Macacus No. 24 was injected intracerebrally with 1 c.c. of the same emulsion. This animal exhibited paralysis of the arm 6 days after the inoculation and died on the 7th day. Animals that have survived an attack of the experimental disease are supposed to have a very high degree of immunity, yet here was such an animal that succumbed almost as readily as the new control animals to an inoculation containing only 3 1/3 times the amount used in the controls. This is additional evidence for the virulence of the material used in the test.

In Charts I and III, it is noted that Macacus Rhesus No. 81 of the five injection series and Macacus Rhesus No. 80 of the ten injection series, had each received a series of five injections of killed virus two and one-half months previously. At that time, we were unable to obtain new monkeys for controlling our test inoculations and for our neutralizing experiments; therefore, the final tests in these animals could not then be made. We thought that if immunity to polio in monkeys lasts no longer than immunity to rabies in rabbits, it would now be well to subject these monkeys to another series of protective inoculations before giving them the test injection.

The virus neutralization table of the five injection series shows that of the three animals bled all give evidence of the presence of neutralization substances in the blood, but in varying degrees of concentration. The serum of No. 81 neutralized the virus completely in the proportion of one to one. The sera of No. 96 and No. 97 extended the incubation period to twenty-one and twenty-five days respectively, whereas in the untreated controls No. 19 and No. 23 the incubation period was four and five days respectively. This prolongation of the incubation period of highly potent virus is to us an indication of the presence of neutralizing substances in the serum. We have been compelled to apply a rather severe test on account of lack of animals. The proportion of 10 parts of serum to 1 of virus has been considered a suitable amount for the determination of neutralizing principles.

How great a quantity of these substances is developed by this process of injection, remains to be determined in later experiments. No normal monkey serum plus virus control was used in these experiments as it has been quite firmly established that the blood of normal monkeys does not contain neutralizing substances for poliomyelitis.

Chart II tabulates the results of another series of tests. In this series, three monkeys were subjected to the five injection method, but in these experiments the inoculations were made up from glycerolated cords only and the intracerebral test inoculation was 0.15 c.c. of the 5 per cent Rockefeller Virus. This change was made with the idea that cord material might possibly produce a higher degree of immunity than a mixture of brain and cord. The test dose was reduced to one-half the amount first used because that amount (0.3 c.c.) appeared to be much more than enough to bring down the control animals.

As indicated in Chart II, two of three animals survived the intracerebral test dose of 0.15 c.c. The third, No. 27, showed first symptoms on the sixth day after the test inoculation. Controls No. 73 and No. 45 each showed first symptoms on the fourth day after the test inoculation.

An additional proof of the potency of the testing virus is given by the fact that monkey No. 10 which had survived an experimental infection from the virus of the 1916 epidemic with a residual paralysis of the right lower limbs, was reinfected fatally with 0.5 c.c. of 5 per cent Rockefeller Virus. Despite the high degree of immunity generally conferred on the animals by recovery from the disease, this animal succumbed readily to re-infection with only three times the dose given the control animals in this series and less than twice the dose used on the controls in the first series.

In the neutralization tests of the sera of the three animals of this series, one serum that from No. 36 completely neutralized the virus in the proportion of one part of serum to one of virus. The sera from No. 27 and No. 66 prolonged the incubation period to twelve and ten days respectively while in the controls No. 73 and No. 45 the incubation period was but four days. It was demonstrated, therefore, as in the first series that the sera from the treated animals contained neutralizing substances but in varying degrees of concentration.

A few further tests were made but not included in the previous tables because only partially carried out. In these experiments three monkeys were subjected to the injection of increasingly virulent material prepared according to the method previously used with a longer interval between doses. (See Additional Experiments.)

Summary of Additional Experiments:—Of these three animals given preventive inoculations, one died of poliomyelitis after a test dose twice as large as the dose used in the first series of tests, one died of generalized tuberculosis with no microscopic lesions of poliomyelitis and the third as the result of accident. In this last there were no microscopic lesions of poliomyelitis, but the serum of the vaccinated animal contained neutralizing substances. None of the three animals showed any harmful effects due to the preventive inoculations.

Summary of Results of 5-Injection Method:—Eleven animals were subjected to injections of the highly potent Rockefeller strain modified by heating for one-half hour at the following temperatures:—

1st dose heated to 55° C.	3rd dose heated to 45° C.
2nd dose heated to 55° C.	4th dose heated to 37° C.

The fifth dose on the fifth day consisted of the unheated 10 per cent. emulsion, 5 c.c. of which were given to each animal. These injections were administered subcutaneously on successive days. Not one of the inoculated animals exhibited any untoward symptoms that were recognizable by careful observation as a result of the course of injections.

Five out of eight of these animals tested intracerebrally resisted successfully the very severe test injection of from three or six fatal doses. The potency of the testing virus in 1910 was such that 0.05 to 1 c.c. of a Berkefeld filtrate was sufficient to produce fatal disease in monkeys. It has been passed through many additional generations since and it is safe to assume that the virulence has mounted considerably in the past eight years. This has recently been confirmed by us. We found that 0.05 c.c. of 5 per cent emulsion was sufficient to kill.

The sera from seven of the above animals tested in the proportion of one part serum to one part 5 per cent virus contained neutralizing substances. Three sera completely neutralized the virus when used in this proportion and four sera prolonged the short four to five day incubation period shown in the controls, to from ten to twenty-five days.

Ten-Injection Series with 10 *per cent Virus Killed by Heat:*—Chart III shows the results obtained with three monkeys vaccinated subcutaneously by daily injections on ten successive days of 5 c.c. of 10 per cent emulsion of Rockefeller Virus heated to 55° C. for one-half hour. Only one monkey, Macacus Rhesus No. 1, survived the intracerebral test injection consisting of 0.15 c.c. of 5 per cent R. V. This was given intracerebrally three weeks after the completion of the course of preventive injections.

On the fourteenth day after the test inoculation, this animal began to show weakness in the legs which progressed slowly so that on January 5, 1918, both arms and legs were completely paralyzed. After this, the progress of the paralysis ceased. On January 10th, the animal was as lively as he could be under the circumstances; the eyes were bright and he eagerly ate fruit that was held up to his jaws. The animal, however, became so infected with body lice that he was etherized to death on January 20th, 1918.

Of the two other test animals, Macacus Rhesus No. 80, died one week after the test intracerebral inoculation of 0.3 c.c. of 5 per cent R. V., and Macacus Rhesus No. 2 died one week after an intracerebral inoculation of 0.15 c.c. of 5 per cent R. V. Macacus Rhesus No. 84, the control for No. 80, also died one week after the intracerebral injection of 0.3 c.c. of 5 per cent R. V. Macacus Rhesus No. 25, the control for Nos. 1 and 2, died one week after the intracerebral inoculation of 0.15 c.c. of 5 per cent R. V. The blood of all three of the animals vaccinated with killed virus, contained neutralizing substances but in varying degree of concentration as shown by the virus neutralization table on Chart III.

Monkeys No. 99, No. 100 and No. 83 were each injected intracerebrally with 0.6 c.c. of a mixture consisting of equal parts of 5 per cent emulsion of R. V. and of serum obtained from monkeys No. 80, No. 1 and No. 2 respectively. Rhesus No. 99 is alive and well. Rhesus No. 100 began to show first symptoms on the 18th day after the inoculation. The next day complete paralysis of both legs developed. This did not progress. At the present time the animal is alive with residual palsies of both lower limbs. Rhesus No. 83 showed first symptoms on the 10th day after the inoculation and died the same day of respiratory paralysis.

The control animals in this series, Rhesus No. 84 and Rhesus No. 25, received intracerebral injections of 5 per cent. R. V. 0.3 c.c. and 0.15 c.c. respectively. The animals died of the infection with respective incubation periods of five and six days.

Summary of Results of Ten-Injection Series:—Of the three animals of this series, two succumbed to the intracerebral test dose as promptly as the controls. The third exhibited paralysis after an incubation period of fourteen days. This animal Rhesus No. 1 recovered, but with complete paralysis of arms and legs. The control animal in this case had an incubation period of 6 days. None of these animals showed any ill effects from the method of preventive injection used.

Of the three sera tested, one that of Rhesus No. 80, neutralized completely in the proportion of 1 part serum to 1 part 5 per cent R. V. Another, that from Rhesus No. 1, delayed the incubation period to 18 days with recovery, but residual paralysis. The serum from Rhesus No. 2 prolonged the incubation period to ten days.

It would seem from the above that the animals subjected to ten subcutaneous protective injections of virus killed by heating at 55° C. for one-half hour, did not offer as great a resistance to the intracerebral test injection of from three to six fatal doses as those that received the 5-injection protective series. In view of the above results, we made a few further experiments to see if we could increase the resistance of the animals by giving seven injections of virus heated to 50° C. for one-half hour and finishing off with three injections of virus heated to 45° C. for one-half hour. Two animals were so injected:

Exp. Macacus Rhesus No. 46:

Jan. 18, 1918.	Received daily subcutaneous injections of 5 c.c. of 10 per cent. R. V. in saline heated to 50°C. for one-half hour, for seven days.
Jan. 26, 1918.	Received for three days daily subcutaneous injections of 5 c.c. of 10 per cent R. V. in saline, heated to 45° C. for one-half hour.
Jan. 29, 1918.	Series of injections completed.
Jan. 30, 1918.	Animal is quiet. Does not feed well. Seems tremulous on moving.
Jan. 31, 1918.	Completely paralyzed.
Feb. 1. 1918.	Autopsied. Sections show poliomyelitis.

Macacus Rhesus No. 7:

Jan. 18, 1918.	Received course of injections similar to that of Rhesus No. 46.
Jan. 29, 1918.	Course of injections completed.
Feb. 6, 1918.	Animal appeared sick. Did not eat. Was quiet. No sign of muscular weakness.
Feb. 7, 1918.	Was found dead in cage. No sign of paralysis had been observed. Autopsy showed pneumonic consolidation of lower right lobe. Section of cord showed slight but unmistakable signs of poliomyelitis.

From this experiment, it is apparent that heating virus to 50° C. for one-half hour does not attenuate sufficiently to exclude the dangerous possibility of infection as the direct result of the injections *per se*. One animal developed frank symptoms of paralysis on the eleventh day after the beginning of the series of injections. The other died nineteen days after the first injection of lobar pneumonia with lesions of poliomyelitis in the cord.

Discussion.

We feel that this work opens up a field for the practical application of specific preventive measures in poliomyelitis. We have subjected 11 monkeys to the five-injection series without any ill effects to the animals as a result of the injections. The possibility of harmful effects from this method if applied to human beings, is of necessity less than when applied to monkeys, inasmuch as this virus has been adapted to the monkey for the past nine years and by continuous passage has become highly virulent for this animal.

The period of time required for this series of injections is a short one, only five days which, of course, would render it highly practicable in time of epidemic when a rapid method is to be desired.

That this method confers a substantial degree of immunity is shown by the resistance to multiple intracerebral doses of this highly potent virus and by the presence of neutralizing substances in the serum. It is a well established fact that persons who recover from poliomyelitis have in their blood neutralizing substances for polio virus and that in all probability the presence of these substances is an indication of immunity to re-infection. The same can be said of monkeys that recover from the experimental disease. It may fairly be stated that animals can be said to have been rendered immune to poliomyelitis by the immunizing processes described since neutralizing substances have been demonstrated in their serum and they have been enabled to resist multiple intracerebral injections of the most virulent material. The disease as it appears in human beings produces a far less serious condition than the intracerebral infection of experimental poliomyelitis in monkeys. In the former case, the body fluids have an opportunity to combat the virus of the infection at the portal of invasion. In the experimental disease, the defensive forces of the body are circumvented. It might, therefore be expected that an animal body containing a large reservoir of these anti-poliomyelitis substances artificially produced, would be able easily to take care of the comparatively mild infection of human poliomyelitis.

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PROTECTION EXPERIMENTS.

1917.

FIVE INJECTION SERIES.

Material Used for Immunization.—A 10 per cent. emulsion of glycerolated brain and cord of R. V. monkeys.

Injections.—5 c.c. subcutaneously on successive days. Each 5 c.c. dose virus heated one half hour on day of injection, as follows: first dose to 55° C., second to 55° C., third to 45° C., fourth to 37° C., fifth dose unheated.

Test Inoculation.—Active virus (see below).

Mac. Rhes.	Preventive Inoculation.	Test Inoculations.		Date Paralysis.		Incubation.	Outcome.	
No.	Date Completed.	Date.	Dose.	Test Animals.	Controls.	Days.	Test Animals.	Controls.
*81	Sept. 18	Sept. 27	0.3 c.c. 5% R.V.	None			Alive—well	
75	None		0.3 c.c. 5% R.V.		Oct. 3	6		Died Oct. 5
90	Nov. 23	Dec. 14	0.3 c.c. 5% R.V.	None			Alive—well	
91	Nov. 23	Dec. 14	0.3 c.c. 5% R.V.	Dec. 20		6	Died Dec. 21	
96	Nov. 23	Dec. 14	0.3 c.c. 5% R.V.	None			Alive—well	
97	Nov. 23	Dec. 14	0.3 c.c. 5% R.V.	Dec. 20		6	Died Dec. 21	
19	None	Dec. 14	0.3 c.c. 5% R.V.		Dec. 18	4		Died Dec. 21
23	None	Dec. 14	0.3 c.c. 5% R.V.		Dec. 19	5		Died Dec. 21
*24	See below	Dec. 14	1.0 c.c. 5% R.V.		Dec. 20	6		Died Dec. 21

* No. 81 had received a similar series of 5 injections June 26 to June 30/17.
* No. 24 had a residual paralysis from experimental poliomyelitis 1½ years before.

CHART I—Continued.

VIRUS NEUTRALIZATION.

With Serum of Monkeys of Above Series.

Mixture of serum and virus kept at 37° C. for two hours, followed by twenty-two hours in ice-box, then injected intracerebrally.

Test Inoculations.				Outcome.							
				Test Animals.				Controls.			
Mac. Rhes. No.	Date.	Material Used.	Dose.	Mac. Rhes. No.	Date Paralysis.	End Result.	Incubation. Days.	Mac. Rhes. No.	Incubation.	Date Paralysis.	End Result.
J	9/27	Serum No. 81 1 part 5% R. V. 1 part	0.6 c.c.	J	None	Alive—well	None	75	6 See above	10/3	Died 10/5
98	12/14	Serum No. 96 1 part 5% R. V. 1 part	0.6 c.c.	98	1/4	Died 1/10	21	19	4	12/18	Died 12/21
								23	5	12/19	Died 12/21
92	12/14	Serum No. 97* 1 part 5% R. V. 1 part	0.6 c.c.	92	1/8	Died 1/10	25	19	4	See above	See above
								23	5	See above	See above

* No. 97 died as result of test inoculation. See table above.

CHART II.

PROTECTION EXPERIMENTS.

1918.

FIVE INJECTION SERIES.

Material Used for Immunization.—A 10 per cent. emulsion of glycerolated cord of R. V. monkeys.

Injections.—5 c.c. subcutaneously on successive days. Each 5 c.c. dose virus heated one-half hour on day of injection as follows:

1st dose to 55°C; 2nd dose to 55°C; 3rd dose to 45°C; 4th dose to 37°C; 5th dose, *UNHEATED.*

Test Inoculation.—Active virus (see below).

Mac. Rhes.	Preventive Inoculations.	Test Inoculations.		Date Paralysis.		Incubation.	Outcome.	
No.	Date Completed.	Date.	Dose.	Test Animals.	Controls.	Days.	Test Animals.	Controls.
36	Jan. 29, 1918	Feb. 19	0.15c.c. 5% R. V.	None		None	Alive Well	
27	Jan. 29, 1918	Feb. 19	0.15c.c. 5% R. V.	Feb. 25		6	Etherized Feb. 26, 1918	
66	Jan. 29, 1918	Feb. 19	0.15c.c. 5% R. V.	None		None	Alive Well	
73	None	Feb. 19	0.15c.c. 5% R. V.		Feb. 23	4		Died Feb. 26, 1918
45	None	Feb. 19	0.15c.c. 5% R. V.		Feb. 23	4		Died Feb. 26, 1918
*10	See below	Mar. 11	0.5c.c. 5% R. V.		Mar. 18	7		Died Mar. 20, 1918

* No. 10 had a residual palsy from experimental poliomyelitis in 1916.

CHART II.—Continued.

VIRUS NEUTRALIZATION.

With Serum of Monkeys of Above Series.

Mixture of serum and virus kept at 37° C. for two hours, followed by twenty-two hours in ice-box then injected intracerebrally.

Test Inoculations.				Outcome.							
				Test Animals.				Controls.			
Mac. Rhes.	Date.	Material Used.	Dose.	Mac. Rhes.	Date Paralysis.	End Result.	Incubation. Days.	Mac. Rhes.	Incubation. Days.	Date Paralysis.	End Result.
4	2/19	Serum No. 36 1 part 5% R. V. 1 part	0.5 c.c.	4	None	Alive—well	None	73	4	2/23/18	Died 2/26/18
								45	4	2/23/18	Died 2/26/18
71	2/19	Serum No. 27 1 part 5% R. V. 1 part	0.5 c.c.	71	3/3/18	Died 3/4	12	73	4	Above	Above
								45	4	Above	Above
16	2/19	Serum No. 66 1 part 5% R. V. 1 part	0.5 c.c.	16	3/1/18	Died 3/2	10	73	4	Above	Above
								45	4	Above	Above

CHART III.

PROTECTION EXPERIMENTS.

1917.

TEN INJECTION SERIES.

Material Used for Immunization.—A 10 per cent. emulsion of glycerolated brain and cord of R. V. monkeys.

Injections.—5 c.c. subcutaneously on successive days. Each 5 c.c. dose heated to 55°C. for one-half hour on day of injection.

Test Inoculations.—Active virus (see below).

Mac. Rhes.	Preventive Inoculations.	Test Inoculations.		Date Paralysis.		Incubation.	Outcome.	
No.	Date Completed.	Date.	Dose.	Test Animals.	Controls.	Days.	Test Animals.	Controls.
*80	Sept. 14, 1917	Oct. 7	0.3 c.c. 5% R. V.	Oct. 13		6	Died Oct. 14	
84	None	Oct. 7	0.3 c.c. 5% R. V.		Oct. 12	5		Died Oct. 14
1	Nov. 30, 1917	Dec. 21	0.15 c.c. 5% R. V.	Jan. 3,'18 Arms & Legs		13	Recovered Etherized Jan. 20, 1918	
2	Nov. 30, 1917	Dec. 21	0.15 c.c. 5% R. V.	Dec. 27		6	Died Dec. 28, 1917	
25	None	Dec. 21	0.5 c.c. 5% R. V.		Dec. 27	6		Died Dec. 28

* No. 80 had received series of 5 injections of 10 per cent. R. V. heated to 55°C. for one-half hour June 26-30th, 1917.

CHART III—Continued.

VIRUS NEUTRALIZATION.

With Serum of Monkeys of Above Series.

Mixture of serum and virus kept at 37° C. for two hours, followed by ice-box for twenty-two hours, then injected intracerebrally.

Test Inoculations.				Outcome.							
				Test Animals.				Controls.			
Mac. Rhes.	Date.	Material Used.	Dose.	Mac. Rhes.	Date Paralysis.	End Result.	Incubation. Days.	Mac. Rhes.	Incubation. Days.	Date Paralysis.	End Result.
99	10/7	Serum No. 80 1 part 5% R. V. 1 part	0.6 c.c.	99	None	Alive—well	None	84	5	10/12	Died 10/14
100	12/22	Serum No. 1 1 part 5% R. V. 1 part	0.6 c.c.	100	1/9/18	Residual paralysis of legs	18	25	6	12/27	Died 12/28
83	12/21	Serum No. 2 1 part 5% R. V. 1 part	0.6 c.c.	83	12/31/17	Died 12/31/17	10	25	6	12/27	Died 12/28

ADDITIONAL PROTECTION EXPERIMENTS PARTIALLY CARRIED OUT.

EXPERIMENT 1.—MACACUS RHESUS No. 57.

Material Used.—Increasingly virulent R. V. in 5 per cent. emulsion.

Injections.—Doses, heating and intervals as follows:

1st dose, Feb. 13, 1917. 10 c.c. heated to 55°C. for one-half hour.
2nd dose, Feb. 16, 1917. 10 c.c. heated to 45°C. for one-half hour.
3rd dose, Feb. 20, 1917. 5 c.c. heated to 37°C. for one-half hour.
4th dose, Feb. 24, 1917. 5 c.c. unheated.

Animals not bled for determination of neutralizing substances. Apparently well till time of intracerebral test injection.

Preventive Inoculations Completed.	Test Inoculation.		Date Paralysis.	Incubation Days.	Outcome.
	Date.	*Dose.			
Feb. 24, 1917	Mar. 20, 1917.	0.6 c.c. 5% R. V.	**Partial Mar. 26 Complete Mar. 27	6	Etherized. Cord-Lesions of Polio.

* Twice dose used in the series shown on Chart I.
** Left hind leg weak. No attempt to stand. Has the "trembles."

EXPERIMENT 2.—MACACUS RHESUS No. 94.*

Material Used.—Increasingly virulent R. V. in 10 per cent. emulsion.
Injections.—5 cc. given subcutaneously.
Heating and intervals as follows:

1st dose April 20, 1917. Heated to 55° C. for one-half hour.
2nd dose April 23, 1917. Heated to 45° C. for one-half hour.
3rd dose April 25, 1917. Heated to 37° C. for one-half hour.
4th dose April 30, 1917. Unheated.

On May 6, 1917, animal died.
Postmortem showed generalized pulmonary tuberculosis.
Cord showed no microscopic lesions of poliomyelitis.

EXPERIMENT 3.—MACACUS RHESUS 100.

Material Used.—Increasingly virulent R. V. in 10 per cent. emulsion.
Injections.—5 cc. given subcutaneously.
Heating and intervals as follows:

1st dose May 10, 1917. Heated to 55° C. for one-half hour.
2nd dose May 11, 1917. Heated to 55° C. for one-half hour.
3rd dose May 12, 1917. Heated to 45° C. for one-half hour.
4th dose May 14, 1917. Heated to 37° C. for one-half hour.
5th dose May 15, 1917. Unheated.

Virus Neutralization.

With Serum of Macacus Rhesus No. 100.*

Mixture of Serum and Virus kept at 37° C. for two hours, followed by twenty-two hours in ice box, then injected intracerebrally.

Mac. Rhes.	Date.	Material Inoculated.	Dose.	Result.
51	June 1/17	Serum No. 100, 1 Part 5% R. V., 1 Part	0.4 c.c.	Alive—Well.

* Animal appeared well until May 29, 1917, when blood was drawn in order to test, for neutralizing substances. As the result of the cardiac puncture the animal died. Postmortem showed hemopericardium. Microscopic examination of cord showed no lesions of poliomyelitis.

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* Animal used had been inoculated some months before with an emulsion of rat fleas from houses where there had been cases of poliomyelitis. No symptoms had developed as the result of this injection, but the animal had not been well for some time before the present experiment. Had been coughing and was somewhat emaciated.

ACTIVE IMMUNIZATION AGAINST DIPHTHERIA IN A LARGE CHILD CARING INSTITUTION.*

By Julius Blum.

During the past few years our ability to detect antitoxin immunity in diphtheria has been greatly simplified by the introduction of the Schick test. Since this test has been shown to be a very accurate measure of the body's antitoxic immunity we are now able by its use to measure the value of any new form of prophylactic treatment which depends on the production of antitoxin. A new form of preventive treatment is the toxin-antitoxin injection first advocated by Theobald Smith and William H. Park, and first actually employed in human beings by von Behring. During the past five years we have been able to use this active immunization and to control it by means of the Schick test in the Home for Hebrew Infants. This institution is especially adapted for this work as the same children can be observed and retested for a period of about five years; furthermore, the ages of the children coincide with the most frequent age incidence of diphtheria.

During the year 1915, diphtheria had been endemic in the institution and there were six deaths due to the disease. It was a problem therefore, to attempt to reduce if possible the morbidity and consequent mortality. We welcomed the opportunity to separate, by means of the Schick tests, those immune from those susceptible, and to attempt to increase the number of the former by means of toxin-antitoxin injections. The diphtheria toxin used in the Schick test was obtained in bulk from the Research Laboratory of the New York City Department of Health. Dilutions for the test were made from the pure diphtheria toxin as follows: One hundred times the minimum lethal dose for the guinea pig was diluted up to 10 cc. with normal saline. This primary dilution does not deteriorate appreciably for a period of two weeks if kept cold in the ice box. The final dilution, good for twenty four hours only, is made by taking 1 cc. of the primary dilution and adding 99 cc. of normal saline. The amount injected intracutaneously for the Schick test is 0.2 cc. of the final dilution, representing 1/50 minimum lethal dose for the guinea pig. The injection is best made on the anterior surface of the forearm in its upper third, using a one cc. record syringe, with a fine platinum iridium needle. Schick injects 1/50 minimum lethal dose for the guinea pig in only 0.1 cc of normal saline. The use of 0.2 cc. of the final dilution is preferable because the area of the reaction is greater in extent and the chance of error in the amount injected is less. In borderline cases the Schick method gives the stronger reaction .

A negative Schick test denotes that there is sufficient antitoxin in the tissues to neutralize the diphtheria toxin injected and therefore enough to protect the individual. A strongly positive Schick test denotes that there is no antitoxin in the tissues and therefore the individual is not protected by antitoxin. Bactericidal substances may, however, be present. Therefore, the person is probably but not certainly susceptible. A moderately positive reaction indicates a faint trace of antitoxin, The positive reaction appears usually in twelve to twenty-four hours, but may be delayed another twenty-four to forty-eight hours. It is characterized by a circumscribed area of redness and slight infiltration at the site of the injection, measuring from one to two centimeters in diameter. This persists usually for at least seven to ten days and then fades with superficial scaling and a dark brown pigmentation. I have seen the pigmentation persist for as long as three to four months. The positive reaction is dependent upon the local destructive action of diphtheria toxin on the tissue cells in the absence of antitoxin. The moderately and faintly positive reactions which occur in those having faint traces of antitoxin are characterized by lesser degrees of redness and infiltration than the strongly positive. The pseudo reaction, a pure sensitization phenomenon occurring in older children and adults, was rarely seen in the young children tested.

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* Published in Am. Jour., Dis. Children, 1920, 20, p. 22.

For convenience the strongly positive reaction can be designated as + the moderately and slightly positive as ± and the very faintly positive as ⩲ Children giving ± and ⩲ reactions have been included in the table with the positive cases. In this institution, for the sake of greater safety they were in all cases given actively immunizing toxin antitoxin injections.

The following table summarizes the reactions obtained in children of various age groups from one month to six years. For comparison I have appended tables showing Schick's and Zingher's results with the Schick test:

RESULTS AT HOME FOR HEBREW INFANTS.

Age.	Total.	Negative Schick (—)	Positive Schick (+±⩲)	Percentage of Positive Schick Tests.
1 to 6 months	97	76	21	21.6
6 months to one year	115	43	72	62.6
1 to 2 years	221	108	113	51.1
2 to 4 years	409	258	151	36.9
4 to 6 years	234	185	49	20.9
Total	1,076	670	406	37.7

ZINGHER'S RESULTS.

Age.	Total.	Negative Schick.	Positive Schick.	Percentage of Positive Schick Tests.
1 to 6 months	101	82	19	18.8
6 months to 1 year	84	40	44	52.3
1 to 2 years	52	28	24	46.1
2 to 4 years	127	82	45	35.4
4 to 6 years	108	88	20	18.5
6 to 8 years	25	22	3	12.0
Total	497	342	155	31.1

SCHICK'S RESULTS.

Age.	Total.	Negative Schick.	Positive Schick.	Percentage of Positive Schick Tests.
New born	291	275	16	5.0
First year	42	24	18	43.0
2 to 5 years	150	55	95	63.0
5 to 15 years	264	133	131	50.0
Total	747	487	260	34.8

As stated in the table, 37 per cent of the children tested at the Home for Hebrew Infants were found to be susceptible in all probability to diphtheria. These susceptible children were most frequently encountered between the ages of six months and four years. They were fewest in number in infants younger than six months or older than four years. The infrequency of positive reactions during the first half year of life is due probably to an immunity inherited from the mother. That this immunity may be lost gradually by the infant was well illustrated by retesting the infants from time to time. It was found that a number of those who gave negative reactions during the first two years of life became positive later on; in other words, their inherited passive antitoxin immunity was gradually lost. Of a total of 119 children under one year of age giving negative tests, 19 infants (or 16 per cent) became positive three to six months later. When first tested ten of these were in their first half year of life and nine in the second half year. Thirteen of 108 negative children (or 12 per cent) became positive during the second year and five of 125 negatives (or 4 per cent) became positive during the third year of life. The following table will illustrate this loss of inherited immunity.

RESULTS OF RETESTING NEGATIVE SCHICK CASES.

Age.	Negative Tested.	Change to Positive Within 6 mos.	Percentage of Cases Changing to Positive.
1 month to 1 year	119	19	16.0
1 to 2 years	108	13	12.0
2 to 3 years	125	5	4.0

The tests in certain families on members under three years of age are also of interest. Of 72 families tested, two or more members gave the same Schick test in 55 families. Variations occurred in 17 families as follows: In thirteen instances the younger member gave the positive Schick test and the older the negative; in four instances the younger member, (in each case under one year of age), gave the negative Schick test, while the older gave the positive. This last variation is undoubtedly due to the disappearance of a passively transmitted immunity. These variations in the negative Schick tests during the first years of life emphasize the importance of frequently retesting infants in institutions. The retests should be done at least every three months.

It is worthy of note that when diphtheria was epidemic at the institution in 1915, in not a single instance did a child with a negative Schick test contract the disease. This record was uninterrupted until January and February 1920, when influenza was epidemic. At that time non-specific croup in contra-distinction to diphtheria croup was a rather frequent complication of influenza. Diphtheria antitoxin was administered to the croupy children having a positive Schick test and all recovered except one. This child died possibly of a complicating laryngeal diphtheria since the throat culture was positive for diphtheria bacilli; but no autopsy was done. Diphtheria antitoxin was adminstered to two very severe cases of croup and broncho-pneumonia with negative Schick test. One of these cases was intubated and died of broncho-pneumonia. Diphtheria bacilli were not obtained from the cultures. The other patient had ulcerative stomatitis with extension of membrane to the pharynx and larynx and broncho-pneumonia. A bronchos copy was done for diagnostic purposes, and intubation was necessary shortly thereafter. Death followed in a few hours. Repeated throat cultures had been negative for diphtheria bacilli; cultures taken directly from the larynx and from a bit of tracheal exudate were positive. The facts that the child had given a negative Schick test and that a large dose of antitoxin had no effect, cause us to believe that the bacilli found in the trachea were not inciting the disease.

Having ascertained that 37 per cent of the inmates of our institution were susceptible to diphtheria, we proceeded to attempt to immunize these 252 children as follows: the slightly or moderately positive, and the strongly positive cases received three injections; the faintly positive two injections only. The injections were given subcutaneously at intervals of one week, in the outer arm at the insertion of the deltoid muscle. Children under one year received 0.5 c.c. and those over one year of age, 1 c.c. of the diphtheria toxin-antitoxin mixture at each injection. There was occasionally a little redness and swelling at the site of the injection and a rise in temperature in twenty-four hours up to 101 deg. to 102 deg. F., coming down to normal in another twenty-four hours. Vomiting occurred in one case and collapse in another with immediate recovery.

In order to test the results the children who had received the above treatment were subjected to the Schick test at two month intervals. Of these children, 252 in number, all of whom had been positive to varying degrees before injection, a large proportion were found to have become subsequently negative. The immunity developed in the children at different intervals; so that the later tests show a larger per cent immune.

SCHICK RETESTS ON 252 POSITIVE CHILDREN AFTER IMMUNIZING WITH DIPHTHERIA TOXIN-ANTITOXIN MIXTURES.

Time Interval After Injection.	Number Tested.	Results.		
		Positive.	Negative.	
Group 1. 3 to 4 months	252	68	184	73%
Group 2. 9 to 10 months	130	12	118	91%
Group 3. 22 months	50	0	50	100%

Of the 252 children originally tested, only 130 remained in the institution at the end of 9-10 months and of this number only 50 remained at the end of 22 months.

From the above table it will be seen that very striking results were obtained. In three to four months after toxin-antitoxin injections 77 per cent of the positive cases had become negative. In the group of fifty cases which were followed up as late as twenty-two months after injection, 100 per cent had become immune. These marked results show the great practical importance of the active immunization of individuals susceptible to diphtheria, whether they are inmates of institutions or at large in the community. In this way an institution can be rendered practically immune. These results also emphasize the possible permanence of the immunity conferred, lasting perhaps during the life of the individual.

It is interesting to note that during a period of two years, there was not a single case of diphtheria at the Home for Hebrew Infants. During this same period there were only about six positive diphtheria cultures in all throat and nose conditions. That this is mere coincidence is possible, but one may draw the conclusion that the toxin-antitoxin injections were probably instrumental in freeing the institution of diphtheria despite the fact that for a time there were present a small number of susceptible children not immunized. It is also possible that the toxin-antitoxin injections rendered the nose and throat an unfavorable site for the growth of diphtheria bacilli. The blood being antitoxic, the local toxin produced by the presence of the bacilli would not irritate the epithelial cells and thus the oral and nasal mucous membrane would be rendered unfavorable for the growth of diphtheria bacilli.

CONCLUSIONS

1. Schick tests made on 1,076 children in an institution during a period of five years show that a negative Schick test is evidence of the presence of sufficient antitoxin in the tissues to protect the individual against diphtheria infection.

2. The moderate or slightly positive tests (±) denote an insufficient amount of antitoxin in the tissues to protect the individual. The tests called very faintly positive ($\underline{\pm}$) show possibly sufficient antitoxin to protect; however it is possible that the transfer of virulent organisms to these individuals may cause them not only to become carriers but to suffer from a superficial infection without any constitutional toxaemia.

3. Tests made on 72 families showed that the younger members of the same family usually gave corresponding reactions to the Schick test. Where variations did occur the younger member gave usually the positive and the older the negative reaction. Infants under six months, because of the transmitted immunity of the mother, were exceptions to this rule.

4. Changes in the Schick reaction from negative to positive during the first three years of life, due to the loss of an inherited immunity, were found to be sufficiently numerous to justify retesting during this period at intervals of a few months. This procedure is especially desirable in infant asylums.

5. The full duration of the active immunity conferred upon individuals susceptible to diphtheria by the injection of toxin-antitoxin is not yet ascertained. In one series of cases the immunity persisted in 100 per cent of a group of 50 children for at least 22 months. Workers in the Research Laboratory have found that the immunity once acquired lasts for at least four and one half years.

6. It is possible to render a child caring institution diphtheria free. This has been accomplished for a period of five years at the Home for Hebrew Infants by injecting all susceptibles with toxin-antitoxin mixtures

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THE SCHICK TEST IN POLIOMYELITIS, SCARLET FEVER, MEASLES AND IN NORMAL CHILDREN.*

ITS BEARING ON THE QUESTION OF NATURAL IMMUNITY

Abraham Zingher.

The routine employment of the Schick test in children admitted to the Willard Parker Hospital with poliomyelitis during the recent epidemic established a most interesting and significant fact. In conjunction with some previously published observations on the Schick reaction in normal children and in children suffering from scarlet fever together with some more recent observations with the test in cases of measles, this may throw some light on the still open question of natural immunity and susceptibility of individuals to infectious diseases.

From a previous communication on the Schick reaction[1] the accompanying tables (Tables 1 and 2) are taken.

TABLE 1.

SUMMARY OF SCHICK TESTS IN 2,700 NORMAL CHILDREN.

Age, Years.	Total.	Negative Schick.	Positive Schick.	Per Cent. Positive Schick.
2 to 4	62	42	20	32.2
4 to 6	318	236	82	25.8
6 to 8	444	347	97	21.8
8 to 10	597	462	135	22.6
10 to 12	584	459	125	21.4
12 to 14	506	416	90	17.8
14 to 16	189	158	31	16.4
	2,700	2,120	580	21.4

TABLE 2.

SCHICK REACTIONS AMONG SCARLET FEVER PATIENTS.

Age, Years.	Total.	Negative Schick.	Positive Schick.	Per Cent. Positive Schick.
½ to 1	10	5	5	50.0
1 to 2	68	22	46	67.6
2 to 4	273	93	180	65.9
4 to 6	249	116	133	53.4
6 to 8	196	123	73	37.2
8 to 15	237	171	66	27.8
Over 15	167	126	41	24.5
	1,200	656	544	45.3

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* Published in Amer. Jour. Dis. Children, 1917, 13, p. 247.

[1] Park, W. H., and Zingher, Abraham: Diphtheria Immunity. Its Determination by the Schick Test. Am. Jour. Pub. Health, 1916, 6, 431.

The tables show the results obtained with the test on a large series of normal children and scarlet fever patients. It is interesting to note that normal children between the ages of 2 and 4 years show about 32 per cent. of positive reaction, as compared with scarlet fever patients, of whom about 65 per cent., or more than twice as many children, gave a positive reaction.

TABLE 3.

THE SCHICK TEST IN MEASLES CASES.

Age, Years.	Total.	Negative Schick.	Positive Schick.	Per Cent. Positive Schick.
Up to 1	35	25	10	28.6
1 to 2	53	35	18	34.0
2 to 4	64	37	27	42.2
4 to 6	44	29	15	34.1
6 to 8	17	14	3	17.6
Total	213	140	73	34.3

Table 3 gives the results obtained with the Schick test in a series of 213 cases of measles. This table shows that between the ages of one and four years there were only about 10 per cent. more positive reactions in children with measles than in normal children of the same age group.

Table 4 shows the results with the Schick test in 1,350 poliomyelitis cases. Between 1 and 4 years of age there is an average of over 80 per cent. of positive reactions. This percentage is based on over 1,000 cases and can, therefore, be accepted as being fairly accurate. It was interesting and striking to see in the wards of the Willard Parker Hospital row after row of children who gave a positive Schick reaction. Indeed, at one time the number of positive tests in this age group reached between 90 and 95 per cent.

What are the significant facts to be found in the four tables? They give the results of tests in a fairly large series of cases and are, therefore, probably correct. Such a preponderance of individuals susceptible to diphtheria among cases of poliomyelitis and scarlet fever suggests one of two possible causes:

TABLE 4.

THE SCHICK TEST IN POLIOMYELITIS CASES.

Age, Years.	Total.	Negative Schick.	Positive Schick.	Per Cent. Positive Schick.
Up to ¼	11	9	2	18.2
¼ to ½	30	20	10	33.3
½ to 1	150	32	118	78.7
1 to 2	529	92	437	82.8
2 to 4	425	88	337	79.3
4 to 6	122	42	80	65.6
6 to 8	42	19	23	54.8
8 to 10	16	11	5	31.3
10 to 15	7	4	3	42.9
Over 15	18	12	6	33.3
Total	1,350	329	1,021	75.6

1. A destruction of the natural diphtheria antitoxin content during an attack of poliomyelitis or of scarlet fever. In order to explain the

greater prevalence of diphtheria among scarlet fever children, von Behring[2] and others have suggested that there is a temporary loss of the natural immunity to diphtheria during the acute febrile stage of the disease. This loss is caused, according to von Behring, by a destruction of the small amount of natural antitoxin present in some individuals, which is just sufficient to give a negative Shcick test. This destruction of the natural antibody content during an acute infectious disease has been claimed for measles as well as for scarlet fever. Karasawa and Schick,[3] however, have shown that the natural antitoxin content in normal children, and later in the same children during an attack of measles, was approximately the same, thus demonstrating that there is no excessive destruction of antibodies against diphtheria during the febrile stage of measles. We had the opportunity of making an interesting observation bearing on this question in cases of scarlet fever and poliomyelitis. Some of the children suffering from these diseases, who gave a positive Schick test on admission to the hospital and were not immunized either actively or passively against diphtheria, continued to show a positive reaction during their entire stay in the institution, which varied from six to eight weeks. If the positive Schick reaction had indicated only a temporary loss of the natural antitoxin during the febrile stage of the disease, then these antibodies would have rapidly reformed during convalescence and the retest with the Schick reaction would have been negative. The continued positive reaction rather points to the following possibility:

2. *A susceptibility to one of the less contagious diseases indicates that the child is also more apt to be susceptible to other contagious and infectious diseases.* From the little that is known of the infectiousness of poliomyelitis, we have in it a disease which is according to Frost[4] only one-fifteenth as infectious as scarlet fever. We should expect, therefore, if the above hypothesis be correct that the relatively small proportion of children who are susceptible to poliomyelitis would be also more apt to be susceptible to diphtheria than children suffering either from scarlet fever or measles. Again, since scarlet fever is only about one-fourth as contagious as measles, we should expect more susceptibility to diphtheria in scarlet fever than in measles patients. In measles on the other hand, we have according to Herrman[5] a disease which is almost universally contagious (96 per cent.) for children between the ages of 1 and 4 years. We should, therefore, expect to find that in patients with measles there would be approximately the same number of children susceptible to diphtheria (positive Schick reactions) as among normal children. The four tables give rather strikingly corroborative proof of the probable correctness of this view.

These results do not, of course, explain the underlying and all-important question relating to the cause and nature of the so-called natural immunity. They lead, however, to the following suggestions:

1. Natural immunity can be best studied in a disease like diphtheria.

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[2] Von Behring, E.: Ueber ein neues Diphtherieschutzmittel, Deutsch. med. Wchnschr., 1913, 39, 873.

[3] Karasawa, M., and Schick, B.: Ueber den Gehalt des Serums diphtherie und masernkranker Kinder an Schutzkörper gegen Diphtherietoxin, Jahrb. f Kinderh, 1910, 72, 460.

[4] Frost, W. H.: Epidemic Poliomyelitis. What Is Known of Its Cause and Modes of Transmission, Pub. Health Rep., 1916, 31, No. 28.

[5] Herrman, C.: The Communicability of Acute Poliomyelitis; Its Spread and Control, Am. Med., 1916, 11, 713.

for which we have a natural antibody that can be exactly measured in the animal test[6] and clinically determined by the Schick test.

2. The presence or absence of natural immunity, as shown by the Schick test, could be profitably studied in its relation to the Mendelian law. By making careful observations on a larger series of families including the children, their parents, and possibly the grandparents, some of the laws controlling the hereditary character of natural immunity might be revealed. To avoid errors, a history of diphtheria or the recent administration of antitoxin, should always be carefully inquired into and noted with each individual.

We drew attention in our very first communication[7] to the fact that there is a probable hereditary element in the natural immunity against diphtheria. By natural immunity is not meant the short immunity present during the first few months of an infant's life. This immunity is only a temporary passive protection caused by the presence of antitoxic antibodies which are probably derived through the placenta from the mother's circulating blood. We pointed out that there is a distinct relationship and grouping among children belonging to the same family. This is shown as follows: (a) the Schick test applied to the children in one family is frequently either uniformly positive or negative in all; (b) where variations exist in the test, the older children generally give the negative, the younger ones the positive reaction; (c) where the youngest child, if more than two years old, gives a negative Schick test, then the older children generally also show a negative test. These interesting phenomena, seem the result of living under the same conditions of environment and the consequent development of an immunity through similar exposures, or else they may be due to to an inherited family tendency to acquire early in life a natural immunity. This latter view is probably corroborated by the following observation. (d) Where the oldest child in a family gives a positive reaction, the younger children with very few exceptions also give positive Schick reactions. It is difficult to conceive that in these families some of the older children had not been exposed and probably frequently exposed, to infections with the Klebs-Loeffler bacillus. This can, therefore, only be explained by the absence of an inherited ability to develop an early immunity to diphtheria.

Summary And Conclusions

1. Between one and four years of age the Schick test is positive in about 32 per cent. of normal children, in a slightly larger proportion of children with measles, in twice as many cases of scarlet fever and nearly three times as many cases of poliomyelitis.

2. Susceptibility to one of the less contagious diseases like poliomyelitis seems to indicate that the child is more apt to be susceptible to other contagious and infectious diseases.

3. Natural immunity reveals interesting problems, which can be partly solved by a study with the Schick test in diphtheria immunity.

4. A uniform technic of making the test, standard solutions of toxin, a uniform interpretation of the test, and a large series of cases are essential in any attempt at making important deductions from the results obtained with the Schick reaction.

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[6] Zingher, A.: A Modification of Roemer's Intracutaneous Method for the Determination of Small Amounts of Diphtheria Antitoxin in Blood Sera, Jour. Infect. Dis., 1916, 19, 557.

[7] Park, W. H., Zingher, Abraham, and Serota, Harry M.: The Schick Reaction and Its Practical Applications, Arch. Pediat., 1914, 31, 481.

ACTIVE IMMUNIZATION OF INFANTS AGAINST DIPHTHERIA.*

Abraham Zingher.

The presence of diphtheria in civilized communities constitutes an important hygienic problem. It shows the need for a practical and efficient method of *active immunization,* that will protect the child and adult population. Such protection should be applied early in life, so that an efficient active immunity will be produced during that period when the individuals are most susceptible and the disease causes the greatest mortality; that is, from one to five years of age.

A. Vital Statistics On Diphtheria

A short review of the tables furnished by vital statistics in this country, in England and in Germany will help to emphasize the foregoing statement which is quite thoroughly realized by physicians in general but especially by those engaged in public health work.

I. Morbidity Statistics.

TABLE 1.

MORBIDITY AND MORTALITY FROM DIPHTHERIA IN NEW YORK CITY DURING THE PAST FIVE YEARS.

Year.	Morbidity.	Mortality.
1913	14,535	1,333
1914	17,129	1,491
1915	15,259	1,278
1916	13,521	1,031
1917	12,624	1,158
Total for five years	73,068	6,291
Yearly average	14,613	1,258

2. *Mortality Statistics.*

TABLE 2.

MORTALITY FROM DIPHTHERIA IN NEW YORK CITY FROM 1891 TO 1900.

Age.	Number.	Per Cent.
Under 1 year	1,665	9.2
1 to 2 years	4,263	23.0
2 to 3 years	3,817	21.2
3 to 4 years	2,900	16.1
4 to 5 years	1,908	10.6
Under 5 years	14,563	81.5
5 to 10 years	3,052	17.0
10 to 15 years	241	1.3
Under 15 years	17,846	

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* This article utilizes not only the investigations of the writer but also those of Drs. M. C. Schroeder and E. J. Banzhof and the Misses Denny and Alper.

TABLE 3.

TOTAL MORTALITY AND MORTALITY UNDER FIVE YEARS FROM DIPHTHERIA IN NEW YORK CITY FROM 1901 TO 1917.

Year.	Total Mortality.	Under Five Years.	Per Cent.
1900	1,920	1,354	70.0
1901	2,068	1,546	74.7
1902	2,015	1,503	74.5
1903	2,190	1,606	73.3
1904	2,084	1,566	75.1
1905	1,544	1,178	76.3
1906	1,898	1,476	77.7
1907	1,470	1,354	92.1
1908	1,758	1,404	79.8
1909	1,714	1,418	82.7
1910	1,715	1,324	77.2
1911	1,281	1,004	78.3
1912	1,125	873	77.6
1913	1,333	972	72.9
1914	1,491	1,143	76.6
1915	1,278	967	75.6
1916	1,031	795	77.1
1917	1,158	842	72.7
Total	29,873	23,150	77.5

TABLE 4.

MORTALITY FROM DIPHTHERIA IN THE UNITED STATES. U. S. SPECIAL REPORT, 1900 TO 1904.

Age.	Number.	Per Cent.
Under 1 year	3,736	7.0
1 to 2 years	8,001	15.0
2 to 3 years	7,990	14.8
3 to 4 years	7,207	13.5
4 to 5 years	5,989	11.0
Under 5 years	32,923	61.0
5 to 10 years	14,818	27.6
10 to 15 years	3,191	6.0
15 years and up	2,628	5.0
Unknown	87	
Total	53,647	

TABLE 5.

MORTALITY CENSUS FROM REGISTRATION AREA IN THE UNITED STATES FOR THE YEAR 1910.

	Deaths.	Deaths per 100,000 population.
1. Diphtheria	11,512	21.4
2. Measles	6,598	12.3
3. Scarlet fever	6,255	11.6

TABLE 6.

MORTALITY FROM DIPHTHERIA IN THE REGISTRATION AREA OF THE UNITED STATES. U. S. CENSUS 1914 AND 1915.

Year.	Number.	Deaths per 100,000 Population.
1914	11,786	17.9
1915	10,544	15.7

TABLE 7.

AGE INCIDENCE OF 2,711 CASES OF DIPHTHERIA ADMITTED TO BAGINSKY'S CHILDREN'S HOSPITAL IN BERLIN BETWEEN 1890-1897.

Age.	Number of Cases.	Per Cent. of Total of Cases.	Number of Deaths.	Death Rate per 100 Admissions.	Death Rate at Each Age Period.
0 to 6 months	15	0.55	4	0.15	26.6
6 to 12 months	69	2.50	36	1.32	52.2
1 to 2 years	227	8.30	110	4.06	48.4
2 to 3 years	317	11.60	119	4.30	37.5
3 to 4 years	354	13.05	121	4.40	34.2
4 to 5 years	337	12.40	84	3.09	24.9
5 to 6 years	264	9.70	61	2.25	23.1
6 to 7 years	280	10.30	61	2.25	21.8
7 to 8 years	209	7.70	26	0.95	12.4
8 to 9 years	175	6.40	23	0.84	13.1
9 to 10 years	146	5.30	19	0.70	13.0
10 to 11 years	101	3.70	9	0.33	8.9
11 to 12 years	80	2.90	8	0.29	10.0
12 to 13 years	65	2.40	7	0.25	10.8
13 to 14 years	72	2.65	6	0.22	8.3
Total	2,711	....	694	....	Av. 25.6

TABLE 8.

MORTALITY FROM DIPHTHERIA IN ENGLAND IN 1915.

	Number.	Deaths per 100,000 Population.
All ages	5,895	16.6
Under 15 years (same age group)	....	49.4

These rates are about the same as in 1914, when the mortality was heavier than in any previous year.

The following facts may be deduced from the tables given:

1. The morbidity and mortality from diphtheria is quite high and constant, showing that (*a*) in spite of the extensive use of small prophylactic doses of antitoxin the total number of cases of diphtheria has not been reduced to any extent; (*b*) the measures taken at present in isolating

and treating the temporary or more persistent carriers of diphtheria bacilli are of little benefit in accomplishing results on a large scale.

2. Children from one to five years of age are most susceptible to the disease and show by far the greatest mortality. In one table 81.5 per cent. of deaths, in another table 77.5 per cent., are shown to have occurred under five years of age. As is well known, the dangerous laryngeal forms and the complicating bronchopneumonias are seen especially in this group of young children.

3. The mortality from diphtheria is nearly as great as that from measles and scarlet fever combined.

4. The yearly mortality from diphtheria in the United States calculated on the basis of the figures given in Table 5 is about 23,540 and the morbidity from this disease would be approximately ten times higher. Such figures present themselves in spite of the very valuable therapeutic remedy which we have in the form of antitoxin It would therefore be of the greatest importance, considering these impressive statistics, to utilize to the fullest extent possible a method of active immunization that will afford our total infant population, as well as the susceptible portion of our child and adult population, protection against this disease.

B. The Schick Test.

Recent investigations into the subject of natural and active immunity in diphtheria,[1] and studies with the Schick test,[2] a valuable clinical test at our disposal at the present time for determining diphtheria immunity, would seem to offer a solution to the problem of the control of diphtheria.

The following tables give the results with the Schick test on various groups of children. Tables 9 and 22 are interesting as showing the very large proportion of positive reactions among children over six months of age.

The protection of the infant derived from the mother is only temporary and lasts about six to nine months after birth. Some children retain this maternal immunity for a year, and some are protected for even one and one-half years. The period of greatest susceptibility to diphtheria is between the first and fifth years of life. After the sixth year the number of chil-

TABLE 9.

RESULTS WITH THE SCHICK TEST.

Age.	Total.	Negative.	Positive.	Per Cent. Positive.
6 mo. to 1 year	10	5	5	50.0
1 to 2 years	68	22	46	67.6
2 to 4 years	273	93	180	65.9
4 to 6 years	249	116	133	53.4
6 to 8 years	196	123	73	37.2
8 to 15 years	237	171	66	27.8
Over 15 years	167	126	41	24.5
Total	1,200	656	544	45.3

See also Table 22, summary of Schick tests at the New York Foundling Asylum.

TABLE 10.

THE SCHICK TEST AT THE NEW YORK CATHOLIC PROTECTORY.

Age.	Total.	Negative.	Positive.	Per Cent. Positive.
4 to 6 years	126	113	13	10.3
6 to 8 years	212	202	10	4.7
8 to 10 years	91	82	9	9.9
10 to 15 years	233	220	13	5.5
15 years and up	25	24	1	4.0
Total	687	641	46	6.7

TABLE 11.

THE SCHICK TEST AT THE INSTITUTE OF MERCY.

Age.	Total.	Negative.	Positive.	Per Cent. Positive.
4 to 6 years	62	54	8	12.9
6 to 8 years	62	52	10	16.1
8 to 10 years	47	41	6	12.7
10 to 15 years	151	141	10	6.6
Total	322	288	34	10.5

TABLE 12.

THE SCHICK TEST AT ST. JOSEPH'S INSTITUTE.

Age.	Total Tested.	Immunes.			Non-Immunes Positive.	Per Cent. Positive.	Per Cent. Pseudo.
		Negative.	Pseudo.*	Total.			
a. (Boys)							
5 to 10 years	55	48	0	48	7	12.7	0.0
10 to 15 years	112	103	8	111	1	0.9	7.1
15 to 20 years	51	39	11	50	1	1.9	21.5
Total	218	190	19	209	9	4.1	8.7
b. (Girls)							
5 to 10 years	36	24	1	25	11	30.6	2.8
10 to 15 years	69	49	11	60	9	13.0	15.9
15 to 20 years	43	28	11	39	4	9.3	25.6
Total	148	101	23	124	24	15.5	16.2

TABLE 13.

THE SCHICK TEST AND CONTROL TEST AT THE DOMINICAN CONVENT.

Age, Years.	Total Tested.	Immunes.			Nonimmunes			Per Cent. Positive.	Per Cent. Pseudo.
		Negative.	Pseudo*	Total.	Positive.	Combined.*	Total.		
4 to 6.........	52	29	8	37	14	1	15	28.8	15.4
6 to 8.........	67	46	14	60	6	1	7	10.4	20.8
8 to 10.........	32	13	12	25	4	3	7	21.8	37.5
10 to 15.........	71	42	25	67	2	2	4	5.6	35.2
Total.....	222	130	59	189	26	7	33	14.8	26.5

* A pseudo-reaction (pseudo-negative reaction) indicates that the individual is immune to diphtheria, but is hypersensitive to the diphtheria bacillus protein contained in the test solution. A combined reaction (pseudo-positive reaction) indicates that the individual is susceptible to diphtheria and is also susceptible to the bacillus protein.

dren showing a positive Schick reaction diminishes rapidly. Table 10 shows from 4 to 10 per cent. of positive reactors after the sixth year. Table 11 also shows a relatively small proportion of positive Schick reactions at this age (average 10.5 per cent.). Table 12 shows in a striking way that occasionally in an institution a group of 100 or more children over ten years of age may be found, among whom only one or two will give a positive reaction. This will explain why diphtheria seems to be a relatively rare disease in some institutions, in which children from seven to fifteen years are congregated.

A prolonged experience extending over a number of years has shown to the writer the following important facts in connection with the Schick test and the active immunization with toxin-antitoxin:

1. A negative Schick reaction, when the test has been carried out properly with a suitable diphtheria toxin, indicates quite definitely the presence of immunity to diphtheria. This fact was conclusively demonstrated in our experience at the Willard Parker Hospital. During a period of over four years, more than 1,200 patients suffering from scarlet fever were admitted to that institution. Each one gave on admission a negative Schick reaction. All the tests were made by the same observer, and the patients were carefully watched every day. None of these patients was immunized either actively or passively, and yet, even though from 15 to 20 per cent. became carriers of virulent diphtheria bacilli at one time or another during their stay in the hospital, not a single patient developed a true clinical diphtheria.

2. The negative Schick test is of value, not only in showing an immunity to diphtheria at the time the test is made, but also in indicating in children over two years of age the *development* of a natural immunity, which seems to be *permanent.* This was quite definitely shown by making Schick retests on such children in the institutions in which we had carried on our work in active immunization with toxin-antitoxin. Over 3,000 children who had given negative Schick reactions at the time of the first test, were retested at different intervals during a period of three years and the negative reaction was found to persist in almost all. From 1 to 2 per cent. gave positive reactions at the retest. These children can be regarded as borderline cases who have a small amount of antitoxin which may show variations under different conditions.

3. In children below six to nine months of age, the immunity as shown by negative Schick reactions is only temporary and derived from the mother through the placental circulation. In breast-fed infants antitoxic antibodies are also derived through the breast milk. Almost all these infants sooner or later become susceptible to diphtheria. Those children whose mothers have no immunity of their own are susceptible at birth, while most of the others whose mothers are immune become susceptible from six to nine months after birth. A few infants remain immune at twelve months and an occasional child may remain protected by its maternal immunity even up to the eighteenth month. These facts came out very strikingly in our results at the New York Foundling Asylum. The results and the conclusions to which they lead in regard to the universal immunization of all children from six months to two years of age, will be given further on.

4. The foregoing tables show that an increasing proportion of children gradually develop a natural immunity to diphtheria. In adult life the majority (85 to 90 and even 95 per cent.) have become immune. It is rather important to bear this fact in mind. It shows that by actively immunizing all children from six to twenty-four months and only those children over two years who give a positive Schick reaction, we can render the child population immune to diphtheria and tide it over the period of greatest susceptibility. The following tables show that we can produce a lasting active immunity with diphtheria toxin-antitoxin in from 95 to 100 per cent. of susceptible individuals. This active immunity is proved by the fact that during subsequent exposure to the disease the children remained free from diphtheria and is also shown by our obtaining persistent negative Schick reactions at later retests. This immunity has been found to last over five years, and in a small group whom we have had the opportunity of observing, over six years. These children will be retested further at yearly intervals to determine whether any number lose their active immunity. So far this has not been the case.

C. Active Immunization With Diphtheria Toxin-Antitoxin.

1. *Toxin-Antitoxin Mixtures as Prepared by E. J. Banzhof.*

The toxin-antitoxin mixtures used for immunization should be slightly toxic and represent about 85-90 per cent. of an L+ dose of toxin to each unit of antitoxin. The mixture should be prepared with a diphtheria toxin of such strength that each dose of 1.0 c.c. of the finished mixture will contain at least three almost neutralized L+ doses of toxin. This is the standard mixture given out at the Research Laboratory. Mixtures made from a very strong toxin containing as much as five almost neutralized L+ doses may be used to advantage, but a toxin so weak as to give a mixture containing less than three L+ doses should not be used for toxin-antitoxin preparation.

If it is desired to prepare a relatively small amount of toxin-antitoxin (about one or two liters) for immediate use, the concentrated antitoxin should be diluted first 1:10 with sterile physiological salt-solution to facilitate the exact measurement of the necessary number of units of antitoxin. A slightly toxic mixture representing 85 per cent. of one L+ dose of toxin to each unit of antitoxin should contain one L+ dose of toxin together with 1.17 units of antitoxin. A slightly stronger mixture representing 90 per cent. of an L+ dose of toxin to each unit of antitoxin should contain one L+ dose of toxin to 1.11 units of antitoxin. For example: if an 85 per cent. L+ mixture is to be prepared with a strong toxin, containing 3.3

L+ doses per cubic centimeter (L+ dose = 0.3 c.c.), then the mixture will require 3.3 x 1.17 = 3.861 units of antitoxin to each cubic centimeter of toxin or 3861 units of antitoxin to the liter of toxin. If a 90 per cent. L+ mixture is to be prepared with the same toxin, the amount of antitoxin required for each cubic centimeter of toxin is 3.3 x 1.11 = 3.663 units or 3663 units to the liter. After the addition of the antitoxin to the toxin, the mixture is thoroughly shaken, tested in the usual manner on the following day for sterility and also for potency on guinea-pigs as noted below.

When it is desired to prepare a larger amount of toxin-antitoxin which does not have to be used immediately, we have found it of importance at our laboratory to mix the toxin and the antitoxin in the calculated proportion of about one L+ dose of toxin to one unit of antitoxin. This mixture is allowed to stand overnight. It is quite toxic for the guinea-pig at this stage, 5 c.c. of the mixture causing the death of a guinea-pig on the fourth day. It would also be toxic if used in human beings without further preparation. The mixture is next passed through a Berkefeld filter to insure sterility and stored away in the ice-box for three to four months. It has been found that the filtration and storage have the effect of reducing the toxicity. At the end of three to four months this mixture corresponds by animal test to the unfiltered freshly-made 85 or 90 per cent. L+ mixture and is safe to be used. The mixture should be tested on guinea-pigs immediately after preparation before the Berkefeld filtration and again after the filtration and storage, when it is ready to use.

The toxicity should be carefully determined on four guinea-pigs, two of the animals receiving subcutaneously 1.0 c.c. each, the other two 5.0 c.c. each, of the toxin-antitoxin. No stronger preparation should ever be sent out from laboratories for use in human beings than one fulfilling the following requirements: The animals receiving the smaller dose should show a slight induration, the other animals receiving the larger amounts should show a moderate or marked local induration and late paralysis, but should not die acutely of diphtheria poisoning. If an animal dies before the 5th day, it indicates an excess of free toxin in the mixture which has to be either carefully neutralized with antitoxin or allowed to remain in the ice-box for another two or three months. The mixture should again be tested for toxicity in animals before it is used on human beings.

2. *Relative Value of Various Mixtures of Toxin-Antitoxin.*

Mixtures which are slightly toxic are safe and will give rise to an active immunity. The slightly toxic mixture will show the better antitoxin response without producing any more local or constitutional symptoms than the neutral or over-neutralized mixtures.

If a toxin is over-neutralized and, for example, contains 1½ to 2 units or more of antitoxin to each L+ dose, it will not be sufficiently effective. An active immunity will be produced in only a small proportion of non-immunes. On the other hand, a mixture of toxin-antitoxin which is under neutralized and very toxic is quite dangerous to use on account of the excess of free toxin.

The following table shows the amounts of toxin and of antitoxin, and the percentage of the L+ dose of toxin to one unit of antitoxin in each mixture.

TABLE 14.

MIXTURES OF DIPHTHERIA TOXIN-ANTITOXIN.

Type Mixture.	Amount of Toxin.	Amount of Antitoxin.	Percentage L+ Toxin to 1 Unit of Antitoxin.
1. Slightly toxic............	1 L+ dose	1.11—1.25 units	80–90
2. Neutral.................	1 L+ dose	1.25—1.5 units	65–80
3. Slightly over-neutralized..	1 L+ dose	1.5 —2.0 units	50–65

3. *Dose and Method of Administration.*

The mixture as above described is used undiluted. The dose is 1.0 c.c. injected subcutaneously in the arm at the insertion of the deltoid. The dose is repeated at weekly intervals until three injections have been given. The amount injected is the same for children as it is for adults. In the younger children the local and constitutional symptoms following the injections of toxin-antitoxin are in fact much less marked than in older children and adults.

4. *Determination of the Immunity Response.*

The development of an active immunity is determined with the Schick test at the end of three months. The use of the Schick retest is delayed until this time for, according to our experience, this period is a sufficient interval for the development of an active immunity in all those who will respond to the toxin-antitoxin injections. The number of successfully immunized persons who finally show a negative Schick retest after three injections of toxin-antitoxin is 90-100 per cent., after two injections 80-90 per cent. and after one injection about 60-80 per cent. By reinjecting those who still give a positive Schick reaction at the end of three months with three more doses of toxin-antitoxin, an active immunity may be developed in almost all susceptible persons. In using the Schick test as an index of immunity, it is important not to mistake a pseudo-reaction for a persistent positive Schick reaction. This mistake can be avoided by using the control test with heated toxin at the same time that the Schick test is made.

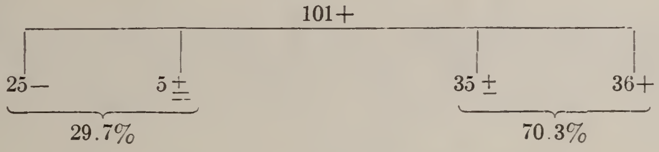
The character of the immunity produced by the injection of toxin-antitoxin may be: (1) an active immunity similar to that following other forms of vaccination; or (2) it may represent the earlier development of a natural immunity that would have more gradually developed in these individuals in later years; or, finally (3) it may represent a combination of these two factors. If it represented only the first type of immunity it would in all probability be only temporary and protect the child for about two or, at the utmost, only three years. If it represented the second type or natural immunity, it would probably be permanent. This is shown by the persistence for years of a negative Schick reaction in those children who are found naturally immune by the test. It is quite likely, however, that we are dealing with the third type, a combination of these two factors. An active immunity is developed and the tissue cells of the individuals are so sensitized that under subsequent conditions of exposure to the ubiquitous diphtheria bacillus, they readily respond by producing a fairly large supply of antitoxin. This remains more or less constant in some, is found increased in others, and in still others is slightly diminished.

5. *Time of Development of Active Immunity After Toxin-Antitoxin.*

Table 15 shows results in a group of 101 children who were admitted to the scarlet fever pavilion of the Willard Parker Hospital. On admission they gave positive Schick reactions and were actively immunized with three doses, each 1.0 c.c. of toxin-antitoxin. A Schick retest was made after three weeks (one week after the third injection of toxin-antitoxin) and subsequently at weekly intervals during the remainder of their stay in the hospital. A certain number of patients were discharged from the hospital at the end of their period of quarantine, so that all children could not be retested for the same period of time (eleven weeks).

TABLE 15.

TIME OF DEVELOPMENT OF ACTIVE IMMUNITY AFTER TOXIN-ANTITOXIN INJECTIONS.



SCHICK RETEST AT THREE WEEKS.

FURTHER SCHICK RETESTS ON THOSE WHO HAD SHOWN A POSITIVE REACTION AT THREE WEEKS.

Number Positives Left.	Time After Injection.	Result. −	±	+
43	4 weeks	2	6	35
19	5 weeks	6	0	13
10	7 weeks	4	0	6
3	9 weeks	1	0	2
2	11 weeks	1	1	0

* Symbols used for Schick reaction.

— indicates a negative Schick reaction and immunity to diphtheria.

± indicates a borderline reaction, in which the individual has probably sufficient antitoxin to give an almost negative reaction. These individuals are grouped among the immunes.

± indicates a moderately positive Schick reaction and susceptibility to diphtheria.

+ indicates a strongly positive Schick reaction and susceptibility to diphtheria.

The table shows clearly that the protection while it developed slowly, was successful in most of the children of this group. It will be seen that at the end of three weeks 30 of the 101 children had become immune and 71 still showed positive Schick reactions. At four weeks, of the 71, 43 were left at the hospital and of these 8 more gave negative reactions. At five weeks, of the 35 non-immunes, 19 were left and of these, 6 gave negative Schick tests. At seven weeks, of the 13 non-immunes, 10 were left and of these, 4 gave negative tests. It will thus be seen that of the non-immunes that we were able to retest, the majority finally showed antitoxin production in from five to seven weeks after the first injection of toxin-antitoxin.

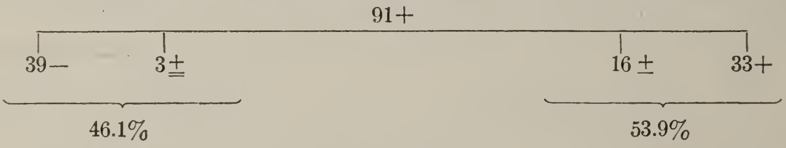
As the active immunity following injections of toxin-antitoxin develops rather slowly such immunization is unsuitable during a sudden, acute outbreak of diphtheria. The danger from the disease to the non-immunes is especially marked when a considerable number of children have been exposed to the infection and have become carriers of virulent diphtheria bacilli. Under such circumstances all positive Schick reactors should be given a prophylactic injection of antitoxin alone. Active immunization could be carried out later (See page 370).

6. *Results of Combined Active and Passive Immunization.*

At two institutions, the Colored Orphan Asylum and the Institute of Mercy, small outbreaks of diphtheria occurred just as the Schick work was started. In consequence of these outbreaks it was necessary to protect the susceptible children at once. We, therefore, gave these children a prophylactic injection of antitoxin. At the Colored Orphan Asylum the children received 750 units of antitoxin in addition to the *first* dose of toxin-antitoxin; at the Institute of Mercy 1000 units of antitoxin were given with the second dose of toxin-antitoxin. It was thought that by this means a rapidly acting *passive* immunity could be given without interfering with the development of the more slowly produced *active* immunity from the toxin-antitoxin injections. In this idea we were mistaken, as we found later.

Table 16.

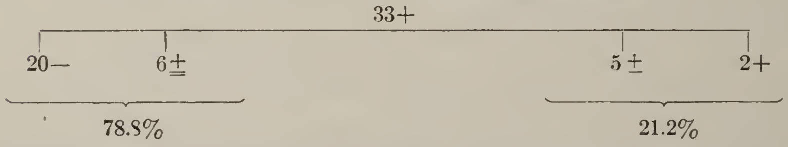
Colored Orphan Asylum, 91 *Susceptible Children.*



Schick retest made at three months. Three doses of toxin-antitoxin, each 1 cc.; 750 units antitoxin with first dose.

Table 17.

Institute of Mercy, 33 *Susceptible Children.*



Schick retest at two and one-half months. Three doses of toxin-antitoxin, each 1 cc.; 1,000 units of antitoxin with the second dose.

As shown in tables 16 and 17, the administration of a prophylactic injection of antitoxin either with the first dose of toxin-antitoxin, as in the Colored Orphan Asylum or with the second dose as in the Institute of Mercy, was sufficient to interfere to a considerable extent with the development of an active immunity.

The results demonstrate that the presence of an injected antitoxin in the body constitutes a distinct interference with the production of an active immunity in response to the toxin-antitoxin stimulation. It is therefore evident that when antitoxin has been used in the routine way to produce a passive immunity no attempt should be made at the same time to produce an active immunity with toxin-antitoxin. An interval of at least two months should be allowed to elapse. Experience has shown that by the end of this time a passively immunizing dose of antitoxin is so far eliminated from the body as not to interfere with the production of an active immunity.

7. Results of Active Immunization in Various Institutions.

Table 18 shows the very successful immunizing results obtained with toxin-antitoxin as determined by subsequent retests with the Schick reaction at intervals of from two and one-half months to four years and four months after toxin-antitoxin injections.

The persistence of the immunity given by the toxin-antitoxin treatment is shown by the fact that with the latest retests all immunized children still remaining in five institutions gave negative Schick reactions four years or more later.

To these results can be added Schick retests made recently on smaller groups of children, (not included in the tables) who were given immunizing injections more than five years ago. In every instance these children gave negative Schick reactions. It is evident, therefore, that the immunity thus conferred is of long duration and may possibly last for life. Persistence in retesting through a long period of years will show whether the latter assumption is correct.

It will be observed that there is a striking contrast between the small percentage of children successfully immunized at the Colored Orphan Asylum and the Institute of Mercy as compared with the high percentage of successful immunization at the other institutions. As previously stated the comparative failure of immunization at these two institutions was due to the prophylactic injections of antitoxin given at the same time with the toxin-antitoxin mixture in consequence of a small outbreak of diphtheria.

TABLE 18.
RETESTS AFTER ACTIVE IMMUNIZATION WITH TOXIN-ANTITOXIN. RESULTS IN VARIOUS INSTITUTIONS.

Institution.	Total Children Tested.	Number Doses of Toxin-Antitoxin.	Interval After Injections.		Schick Results.		Per Cent. Negative on Retest.
			Yrs.	Mos.	Neg.	Pos.	
EARLY RETESTS.							
1. St. Agnes	54	1 or 2	1	0	50	4	92.5
2. St. Dominic	83	2 or 3	1	0	82	1	98.5
	19	1	0	4	13	6	68.4
3. N. Y. Juvenile Asylum	82	3	1	0	80	2	97.8
4. St. Joseph's	55	2 or 3	0	10	55	0	100.0
	18	1	0	10	15	3	84.7
5. House of Refuge	72	2 or 3	0	2.5	66	6	91.6
	56	1	0	2.5	48	8	85.7
6. Hebrew Inf. Asylum	48	*2	0	4	43	5	89.8
	28	*1	0	4	24	4	85.7
7. Hebrew Orph. Asylum	117	1	0	8	95	22	81.2
8. Leake and Watts	43	2 or 3	0	6	43	0	100.0
	7	1	0	2.5	3	4	42.8
9. Colored Orph. Asylum	91	**3	0	3	42	49	46.1
10. Skillman	150	2	0	2.5	142	8	94.6
LATER RETESTS.							
1. St. Agnes	20	1	1	8	18	2	90.0
	25	2	1	8	25	0	100.0
	12	1	0	8	5	7	41.6
2. St. Dominic	41	2	2	1	41	0	100.0
	43	3	2	1	43	0	100.0
3. St. Joseph's	19	2	2	1	19	0	100.0
	39	3	2	1	39	0	100.0
4. Leake and Watts	49	3	1	5	48	1	98.0
	15	3	0	6	11	4	73.3
5. Colored Orph. Asylum	68	**3	1	2	38	20	55.7
6. Institute of Mercy	29	***3	1	0	28	1	96.6
LATEST RETESTS.							
1. St. Agnes (a)	30	1 or 2	4	4	30	0	100.0
2. St. Dominic (a)	23	2 or 3	4	4	29	0	100.0
	10	2	2	7	10	0	100.0
3. St. Joseph's (a)	25	2 or 3	4	0	25	0	100.0
4. Leake and Watts (a)	33	2 or 3	3	6	33	0	100.0
5. Hebrew Orph. Asylum (a)	20	1	4	6	20	0	100.0
6. Hebrew Infant Asylum (a)	30	1, 2 or 3	2	10	30	0	100.0
	11	1, 2 or 3	3	10	11	0	100.0
7. Colored Orph. Asylum	23	**3	3	6	8	15	35.0

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* A few children that had resisted immunization received three more injections of toxin-antitoxin at this time. Schick retest 10 months later, negative in all these children.

** Received 750 units of antitoxin with first dose of toxin-antitoxin.

*** Received 1,000 units of antitoxin with second dose of toxin-antitoxin.

(a) Dr. M. C. Schroeder of the Research Laboratory retested these children during the absence of the writer in service overseas. She had reinjected the few children that had resisted immunization.

D. ANTITOXIN DETERMINATION IN THE ACTIVELY IMMUNIZED

The three tables following represent determinations of the antitoxin content of actively immunized children. They show the amount of antitoxin produced and the variations in examinations made at different times on the same persons.

The amount of antitoxin in Table 19 and in the two tables following represents units of antitoxin per cubic centimeter of serum. The determinations were made in the guinea-pig by a modification[5] of Römer's intradermal test.

TABLE 19.

TWO ANTITOXIN DETERMINATIONS MADE ON ACTIVELY IMMUNIZED CHILDREN SIX MONTHS AND ELEVEN MONTHS AFTER TWO OR THREE INJECTIONS OF TOXIN-ANTITOXIN.

(A) SHOWING A DIMINISHED OR A SIMILAR AMOUNT OF ANTITOXIN AT THE SECOND DETERMINATION.

Number.	Six Months, Units per cc. Serum.	Eleven Months, Units per cc. Serum.
1	2.0	1.0
2	1.5	1.0
3	0.012	0.012
4	0.012	0.01
5	0.2	0.2
6	0.005	0.005
7	0.5	0.2
8	0.5	0.25
9	0.01	0.01
10	0.2	0.2
11	0.5	0.2
12	0.12	0.1
13	0.3	0.3
14	0.12	0.1
15	0.02	0.01
16	0.1	0.05
17	0.02	0.02
18	0.01	0.01
19	0.02	0.01
20	0.01	0.01
21	0.01	0.01
22	0.1	0.01
23	0.012	0.01
24	1.0	0.5
25	0.05	0.05

(B) SHOWING AN INCREASED AMOUNT OF ANTITOXIN AT THE SECOND DETERMINATION.

Number.	Six Months, Units per cc. Serum.	Eleven Months, Units per cc. Serum.
26	1.0	3.0
27	0.05	0.5
28	0.2	1.0
29	0.1	0.2
30	0.1	0.25
31	0.012	0.5
32	0.2	0.5
33	0.1	0.2
34	0.005	0.02
35	0.75	2.0
36	0.1	0.2
37	0.02	0.1
38	0.01	0.1
39	0.01	0.2
40	0.01	0.2

TABLE 20.

RELATIVE EFFICIENCY OF TWO AND THREE DOSES OF TOXIN-ANTITOXIN

Units of Antitoxin in Blood Serum.	Group 1. 2 Doses, Each 1.0 c.c. Children Injected.	Group 2. 3 Doses, Each 1.0 c.c. Children Injected.
0.01	7	9
0.02	2	8
0.05	1	0
0.1	1	6
0.2	3	6
0.5	2	5
1.0	0	5
2.0 up	0	1
Total	16	40
Total showing 0.1 unit and up	6	23
Per cent. showing 0.1 unit and up	37.5	57.5

The results in Table 20 show that a better antitoxin production takes place after three than after two injections of toxin-antitoxin.

The next (Table 21) shows the persistence of the active immunity, as determined by the presence of antitoxin in the blood from fifteen to eighteen months after the injections of toxin-antitoxin. It shows also that a fairly large proportion of the immunized children have a considerable amount of antitoxin even as late as eighteen months after the injections of toxin-antitoxin.

TABLE 21.

ANTITOXIN CONTENT OF ACTIVELY IMMUNIZED CHILDREN FIFTEEN TO EIGHTEEN MONTHS AFTER TOXIN-ANTITOXIN INJECTIONS.

Units of Antitoxin, Per c.c. Serum.	Institution.			Total.
	St. Dominic.	Leake and Watts.	St. Agnes.	
0.005	7	4	..	11
0.01	5	13	..	18
0.02	8	7	..	15
0.05	16	5	..	21
0.1	9	5	7	21
0.2	13	13	1	27
0.5	15	16	13	44
1.0	2	1	13	16
2.0	4	2	7	13
4.0	3	2	9	14
8.0	5	1	0	6
Total	87	69	50	206
Total showing 0.1 unit and up	51	40	50	141
Per cent showing 0.1 unit and up	58.6	60.0	100.0	53.9

E. OBSERVATIONS ON MATERNAL IMMUNITY TO DIPHTHERIA IN YOUNG INFANTS AND ACTIVE IMMUNIZATION WITH TOXIN-ANTITOXIN.

(New York Foundling Asylum).

The following tables represent the results of studies at the New York Foundling Asylum on the duration of maternal immunity in young infants and on the efficiency of the immunization with toxin-antitoxin in infants and mothers who gave a positive Schick reaction.

Table 22 shows that the largest proportion of positive Schick reactions was found between the ages of 6 months and 4 years. The positive reactions diminish rapidly after the fourth year of life.

TABLE 22.

SUMMARY OF SCHICK TESTS BY AGE GROUPS.

Age.	Total	Negative.	Positive.	Per Cent Positive.
0 to 6 months	101	82	19	18.7
6 to 12 months	84	40	44	52.3
1 to 2 years	52	28	24	46.1
2 to 4 years	127	82	45	35.4
4 to 6 years	108	88	20	18.5
6 to 8 years	25	22	3	12.0
Total	497	342	155	31.2

In Table 23 it is interesting to note the relatively small number of pseudo-reactions in children up to 8 years of age (4.4 per cent.) and the relatively large number of adults (38.0 per cent.) as compared with the positive Schick tests of which the children (27.3 per cent.) have nearly three times as many as the adults (9.7 per cent.).

TABLE 23.

SUMMARY OF SCHICK TESTS AND OF CONTROL TEST WITH HEATED* TOXIN ON CHILDREN AND ADULTS.

Age.	Total Tested.	Immunes.			Non-Immunes.			Per Cent. Positive.	Per Cent. Pseudo.
		Negative.	Pseudo.*	Total.	Positive.	Combined.*	Total.		
Children, 0 to 8 years	519	354	23	377	140	2	142	27.3	4.4
Adults	113	59	43	102	6	5	11	9.7	38.0
Total	632	...	...	479	...	...	153	...	...

* See Note, page 369.

TABLE 24.

COMPARATIVE RESULTS WITH SCHICK TEST IN MOTHERS AND INFANTS.

Ages of Infants, Months.	A. Mother Neg. Infant Neg.	B. Mother Neg. Infant Pos.	C. Mother Pos. Infant Pos.	D. Mother Pos. Infant Neg.
Up to 3	18 } 37	1	4	0
3 to 6	19	2	2	0
6 to 9	9	11 } 27	1	0
9 to 12	2 } 8	10	0	0
12 to 15	4	6	0	0
15 to 24	2	2	0	0
Total	54	32	7	0

TABLE 25.

SCHICK TEST AND RETEST AT 6.5 MONTHS IN MOTHERS AND INFANTS. ACTIVE IMMUNIZATION OF SUSCEPTIBLE INFANTS WITH TOXIN-ANTITOXIN.

Group 1 No.	Mother Immune.			Infant Immune.			Retest.	
	Mother.	Test. Feb. 5.	Retest. Aug. 21.	Infant.	Age.	Test.	Age.	Result.
1	A. G.	Ps.—	Ps.—	A. G.	1 mo.	—	7 mo.	+
2	L. W.	Ps.—	Ps.—	F. W.	4 mo.	—	11 mo.	+
3	A. S.	Ps.—	Ps.—	J. S.	5 mo.	—	12 mo.	++
4	B. S.	—	—	A. S.	6 mo.	—	12 mo.	±
5	A. C.	—	—	J. C.	4 mo.	—	11 mo.	+
6	B. C.	Ps.—	Ps.—	R. C.	5 mo.	—	12 mo.	—*
7	M. C.	Ps.—	Ps.—	C. C.	6 mo.	—	13 mo.	+
8	M. L.	—	—	M. L.	5 mo.	—	12 mo.	—
9	C. B.	—	—	H. H.	2 wk.	—	7 mo.	±
10	C. M.	—	—	E. M.	3 mo.	—	10 mo.	+
11	W. F.	—	—	R. F.	2 mo.	—	8 mo.	+
Group 2.	Mother Immune.			Infant Susceptible. Toxin-Antitoxin.			Age.	Result.
12	J. E.	—	—	M. E.	8 mo.	++	14 mo.	—
13	M. R.	—	—	G. R.	8 mo.	+	15 mo.	—
14	S. C.	Ps.—	Ps.—	J. C.	6 mo.	+	13 mo.	—
15	R. K.	Ps.—	Ps.—	E. K.	7 mo.	+	14 mo.	—
16	C. C.	Ps.—	Ps.—	T. C.	6 mo.	+	13 mo.	—
Group 3.	Mother Susceptible. Toxin-Antitoxin.			Infant Susceptible. Toxin-Antitoxin.			Age.	Result.
17	M. M.	+	—	E. M.	1 mo.	+	7 mo.	—
18	G. W.	Ps.+	Ps.—	E. W.	6 wk.	+	7 mo.	—
19	A. W.	Ps.+	Ps.—	A. W.	5 mo.	+	12 mo.	—
20	V. P.	+	—	G. P.	5 mo.	+	12 mo.	—

Ps. — = Pseudo-reaction in an immune mother.
Ps. + = Pseudo-reaction and positive reaction or combined reaction in a susceptible mother.
* Marasmus.

Table 24 shows the following interesting points:

(a) The antitoxin immunity of the infant obtained from the immune mother lasts for about six to nine months after birth. Column A shows that during the first six months of life thirty-seven out of fifty-four, or 68.5 per cent., gave a negative Schick test. The negative reaction in the nine children between six and nine months of age is also due probably to a continuation of the passive immunity derived from the mother, while in the remaining eight children we may or may not be dealing with such an immunity. Only repeated Schick tests at later intervals could determine this point. The children who were still passively protected would lose their antitoxin in the course of the next few months and then give a positive reaction; while those whose negative Schick reaction indicated an early developed natural immunity would continue to show a negative reaction.

(b). Column B shows that a large proportion of children belonging to immune mothers have positive Schick tests after the sixth month of life. Twenty-seven out of thirty-two children between six and fifteen months, or 84.3 per cent, gave a positive Schick reaction.

(c). Column C shows results in seven children who gave positive reactions and whose mothers also gave positive reactions to the Schick test. The results indicate definitely that where a mother has no immunity none will be present in her infant if it is below six months of age.

(d). Column D shows that no case was found in which the mother had a positive and her young infant a negative Schick reaction. It was, of course, to be expected that where the mother had no immunity none would be found in her infant.

Table 25 shows in a striking way the following points:

1. In Group 1 there are eleven mothers and their infants all of whom gave negative Schick reactions at the time of the first test. The age of the infants at this time was six months or under. When retested six and one-half months later, all but two of the infants gave positive Schick reactions. One of the two (No. 6) was marasmic at the time of the retest. No. 4 was slowly losing its maternal immunity at twelve months, No. 7 was beginning to lose it at seven months and had completely lost it at thirteen months. No. 9 was just beginning to lose its maternal immunity at seven months.

This group and the two groups following show that the infant does not inherit the hypersensitiveness to the protein of the diphtheria bacillus shown by the mother in the form of a pseudo-reaction.

2. In Group 2 there are five mothers who were immune and gave negative Schick reactions. Their infants six months of age or over were susceptible and gave positive reactions. The infants were actively immunized with three doses, each 0.5 c.c. of toxin-antitoxin. At the retest six and one-half months later, all gave negative Schick reactions, thus showing that they had been successfully immunized.

3. In Group 3 there are four susceptible mothers whose young infants were also susceptible. All gave positive Schick reactions at the primary test. To the mothers three doses of toxin-antitoxin, each 1 cc. were given; to the infants three doses, each 0.5 cc. At the retest six and one-half months later, both the mothers and their infants gave negative Schick reactions, thus showing that they had all become actively immunized.

4. The table shows very definitely that almost all infants if they are not already susceptible at birth, become susceptible to diphtheria within the first year of life. Only a few infants retain their maternal immunity after

the twelfth month and probably all lose it before the twenty-fourth month of life.

5. The results show the efficiency of the active immunization with toxin-antitoxin.

6. From the table it can also be seen that a large proportion of mothers, ten out of twenty, gave pseudoreactions. Their infants showed straight negative or positive Schick reactions, no pseudoreactions. The susceptibility of the tissue cells to the bacillus protein which gives the pseudoreactions is not transmitted from mother to offspring, but is gradually acquired as the children become older. The cause of this it is probably repeated exposures to and mild infections with the diphtheria bacillus.

The pseudoreactions are best determined[3] by making a control test on the opposite forearm with a dilution of diphtheria toxin which has been heated at 75° C. for five minutes. In the control dilution, 25 per cent more of the heated toxin is used; the excess is added to allow for any possible deterioration that may have taken place in the bacillus protein material during the heating process. It is this protein substance which causes the pseudoreactions.

The toxin-antitoxin injections produced *very little local or constitutional disturbance* in these young infants. It is this freedom from any marked reaction to the injections that makes the immunization in early life a very desirable procedure. Some of the mothers, especially those showing combined reactions, had rather severe local and constitutional symptoms consisting of redness, swelling and tenderness of the arm, headache and slight febrile disturbance, lasting about two days. This susceptibility of older individuals is due as stated before to the bacillus protein contained in the mixture.

F. Observations on the New-Born, Who Were Injected with Toxin-Antitoxin During the First Week of Life.

This important subject was investigated by Miss Helen Denny and Miss Leah Alper under the direction of Dr. Park. There were injected about 2,000 new-born infants at three large Maternity Hospitals in New York City. The Schick test was made on only a few of the infants before the injection of the first dose of toxin-antitoxin. These babies were given from one to three injections, each dose being 1.0 cc. On account of the short post partum stay of the mothers in the hospital, the injections were made closer together than usual, one being given every other day. Practically no local or constitutional symptoms were noted.

TABLE 26.

SCHICK RETESTS* ON YOUNG INFANTS INJECTED WITH TOXIN-ANTITOXIN DURING THE FIRST WEEK OF LIFE.

Number Injections Toxin-Antitoxin.	Total Number of Infants Restested.	Positive Schick.	Negative Schick.	Per Cent. Negative Schick.
1	66	59	7	10.6
2	48	46	2	4.1
3	66	53	13	19.7
	180	158	22	12.3

* Retest made 12 to 18 months after toxin-antitoxin injections.

These results show clearly that injections of toxin-antitoxin in the new-born do not produce an active immunity. This is true even with relatively large doses (1.0 c.c. in the new-born corresponding to 20 times that amount in the adult). The number of infants found to give a negative Schick reaction after the inoculations was only 12.3 per cent. This small proportion of Schick negative children is normally present in this age group. We can assume therefore that the injections of toxin-antitoxin failed completely to produce an immunity response in the new-born infants. The probable cause of this failure may be two-fold: (a) The presence of antitoxin derived normally from the mother through the placenta which gives the infant a passive immunity lasting about six to nine months. The controlling influence of this factor is made more probable by the results noted in the Colored Orphan Asylum (see Table 16). Here children passively immunized with 750 units of antitoxin received also toxin-antitoxin. Of these children, only 35 per cent. developed an active immunity as compared with 100 per cent. in the other institutions where no preliminary antitoxin was given.

(b) The second possibility may be that the tissues of the new-born infant have not yet acquired the ability to produce antitoxin in response to the injection of toxin-antitoxin antigen. Further work is being carried on at the present time to clear up this point.

Another important fact, shown by the table, is the very large proportion of positive Schick reactions found in children of 12 to 18 months of age. This indicates the great need of protecting children of this age group against diphtheria.

G. Observations on the Active Immunization of Infants from Six Months to Two Years of Age (Denny and Alper).

Fortunately, inoculation with toxin-antitoxin gives much better results in infants from six to twenty-four months of age than in the new-born. Our figures show that toxin-antitoxin injections give an active immunity to most children over six months of age. (See Table 25).

TABLE 27.

RESULTS OF TOXIN-ANTITOXIN INJECTIONS IN INFANTS BETWEEN SIX MONTHS AND TWO YEARS OF AGE AT MILK STATIONS.

	Total Children Injected.	Number Injections Toxin-Antitoxin.	Positive Schick Retest.	Negative Schick Retest.	Per Cent. Negative Schick.
Group I Schick Test Positive Before Injections of Toxin-Antitoxin..................	80	3–5	25	55	68.7
Group II Schick Test Negative Before Injections of Toxin-Antitoxin..................	36	2–3	12	24	66.0

Table 27 shows the contrast between the results of immunizing injections in infants over six months of age and in the new-born. Most of these children were under two years at the time of the injection. It will be seen that much better active immunization was obtained at the latter period when the passive immunity derived from the mother is generally lost.

Further work is being continued on this group of children. We feel that the ultimate control of diphtheria will depend to a large extent upon our ability to render immune the child population of this age group.

SUMMARY AND CONCLUSIONS.

1. All infants between six and twenty-four months of age should be actively immunized with three doses, each 1.0 c.c. of toxin-antitoxin. These injections should be given irrespective of the Schick test the infants may show at the time of immunization. The injections are given subcutaneously in the arm or below the angle of the scapula, and repeated every seven days. The toxin-antitoxin is well tolerated by young infants and for that reason the dose advised is the same as that given to older children. The relatively larger dose of toxin-antitoxin will, of course, give rise also to a better immunizing response.
2. Infants below six months of age should not be given the toxin-antitoxin injections for they do not develop an active immunity against diphtheria during the very early months of life. It is probable that the passive antitoxic immunity normally derived from the mother through the placenta interferes with the development of an active immunity in infants below six months of age. It is possible also that the tissues of these young infants have not yet acquired the ability to react to the injections of toxin-antitoxin and so produce antitoxin.
3. All children over two years of age as well as all youths and adults, should be tested with the Schick reaction first and only those giving positive reactions need be immunized with toxin-antitoxin. Three injections, each 1.0 c.c. are given subcutaneously, one week apart.
4. In infants under two years of age the Schick test is not a necessary part of the immunization since *all* the infants between six and twenty-four months should be actively immunized. By omitting the preliminary Schick test the procedure is greatly simplified for many general practitioners to whom the test appears even now as a difficult and complicated procedure. The greater part of the most effective prophylaxis against diphtheria still remains in the hands of the general practitioner. It will be only by getting his interested cooperation in this work and facilitating the process of immunization for him that results of any magnitude will be achieved. Whenever possible, however, it will be of scientific interest to apply the Schick test to infants as well as to older children and adults.
5. The toxin-antitoxin mixture should be prepared in a reliable laboratory and carefully tested for potency in the guinea-pig before it is sent out for use. The mixtures used should be slightly toxic and should represent about 85 to 90 per cent. of an L+ to each unit of antitoxin. It is important that the toxin-antitoxin be supplied free of charge to physicians by municipal and state departments of health, so that immunization on a large scale will be carried out. For the Schick test, the outfit[4] devised by the writer will be found very convenient.

6. Improvements in the method of immunization are possible and further work is being carried out at present to see especially whether two doses of toxin-antitoxin will give in a considerable number of children results as good as those obtained with three doses. This would undoubtedly simplify the process and make it very convenient for general use. Our previous results with one or two doses have not shown so high a percentage of successful immunization as those obtained by three injections.

7. The active immunization against diphtheria should be carried out first of all by the private physicians in the different homes where a majority of the infants can be reached. In larger centers of population the milk stations, day nurseries, children's dispensaries, infant and orphan asylums furnish large groups of children suitable for immunization. The children of pre-school age found in kindergartens and those of school age should be tested first with the Schick reaction and those giving a positive test actively immunized. Whether such procedure can be made compulsory in the schools will depend on the enlightenment of the community in which the work is to be carried out. Adults especially those frequently or constantly exposed to diphtheria such as doctors, nurses, hospital orderlies and patients in contagious disease hospitals, should also be tested with the Schick reaction and those giving a positive reaction should be actively immunized.

8. Finally, it may be stated that the diphtheria problem seems nearer a solution by the thorough application of these newer diagnostic and immunizing procedures which should be encouraged by the various departments of health.

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A CHRONIC AND A NORMAL OR CONTACT CARRIER OF B. TYPHOSUS ASSOCIATED WITH A MILK-BORNE OUTBREAK OF TYPHOID FEVER.*†

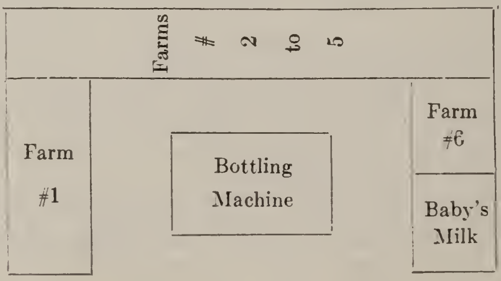
Charles F. Bolduan and Charles Krumwiede, Jr.

Ever since typhoid carriers were first demonstrated by Conradi and Drigalski, many observers have shown that some individuals exposed to infection excrete typhoid bacilli subsequently in their stools without developing any symptoms of disease. These persons are termed "normal" or "contact carriers"; probably most of them are only temporary carriers. However, some undoubtedly become chronic carriers as is evidenced by the existence of chronic carriers with no history of typhoid fever. Many without such history may well have had so mild an infection that it was overlooked or forgotten.

The point of interest in the milk-borne outbreak that we report is that a normal or contact carrier and a chronic carrier were found on one of the farms supplying the milk and the former was at first assumed to be the source of the infection.

The facts concerning the outbreak are briefly as follows: eleven cases of typhoid fever were directly traced to milk from the "B" Dairy. Two additional cases were probably infected from the same source. The dates of onset of the cases extended from October 15th to November 16th. This dairy distributed about 880 quarts of milk a day, obtaining its supply from six farms. About 160 quarts of the milk from one farm were distributed as "Baby's Milk."

The milk received at the dairy was bottled and placed in the ice boxes as shown (see sketch) in the order of the numbers given to the farms supplying the milk. An exception was the "Baby's Milk," a late afternoon milking which was usually bottled on its arrival in the freshly sterilized bottling apparatus.



When the four drivers started out in the morning driver "Peter" took his supply first, usually nearly all of the milk of Farm No. 1. The other drivers then took their supply in the order of the farms given. All took their share of "Baby's Milk" but "Peter" took as much as the other three drivers together.

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* We are indebted to the Board of Health of Morristown, N. J., for the opportunity of studying this outbreak and for permission to report the results.

† Published in the Public Health Reports (U. S. Public Health Service), 1917, 32, p. 1755.

All the cases of typhoid fever were on Peter's route which is evidently explained by his supply coming exclusively from Farm No. 1, the source of the infection as subsequently determined. The contamination of the bottling tank by this milk was evidently not marked as the milk bottled subsequently was not infectious.

One case of typhoid fever was that of a child 1½ years of age who, it was claimed, received "Baby's Milk" only. As the only mark of identification was a pencilled "B" on the cap, accidental substitution of bottles of the ordinary milk was easily possible and probably occurred in this case. The driver, Peter, had worked in the dairy for years and denied all illness both of himself and members of his family.

All the farms were inspected and blood and feces obtained of all individuals at the farms as well as of those working in the dairy. All examinations were negative except those of the specimens from two men on Farm No. 1, S. M. and L. M. The data with regard to these two men at the time of the investigation are as follows.

S. M., employed at the dairy for three months denies ever having had typhoid fever. Widal reaction Nov. 12th, suspicious; Fecal examinations, Nov. 17th, negative, Nov. 26th, negative.

L. M, employed for four months, gives no history of having had typhoid fever. Widal reaction, Nov. 12th, negative. Fecal examinations, Nov. 17th, *positive*, Dec. 2nd, negative.

The above findings coupled with the mode of distribution of the milk seemed to us conclusive evidence as to the source of the infection. This deduction, however, was shown by subsequent events to be erroneous. Although we were not quite satisfied with the Widal result in S. M. the two negative fecal examinations seemed to indicate that the slight Widal reaction was probably due to an exceptionally high content in normal agglutinins, a not infrequent finding.

The carrier L. M. left the farm but we were able to locate him and examined further fecal specimens for a period of six months, none of which were positive. A most rigid inquiry of both L. M. and of his parents failed to elicit any history of an illness which could be considered typhoidal in character.

The helper S. M. left the farm about the same time but returned to work in February. In March we were notified that two cases of typhoid fever had developed, both in children receiving milk directly from Farm No. 1, the milk not passing through the dairy. The fact that S. M. had returned raised our suspicions at once because of his previous Widal record. A fecal examination revealed the presence of typhoid bacilli. Subsequently four other cases of typhoid were reported, all attributable to milk from Farm No. 1. The absence of S. M. and not the exclusion of L. M. was the reason that no cases had occurred after the resumption of distribution by the dairy following our first investigation.

We believe the following to be the interpretation of these unusual findings. The Widal reaction of S. M. indicates that he was a chronic bacillus carrier, but at the time of our investigation was excreting no bacilli or too few to be found by the bacteriological methods employed. Later on his return, he was excreting bacilli more freely and no difficulty was encountered in isolating them. He then disappeared and we could not observe him further. L. M. was a transient normal carrier infected by the milk or more probably through contact with S. M.

We had been in error, therefore, because the carrier found first was a normal carrier, a fact unsuspected at the time. The presence of such a carrier, raises interesting possibilities. Had we relied wholly upon the Widal reaction for the selection of fecal specimens and had S. M. given positive fecal results at the time, we would have excluded him and agreed to the resumption of the milk supply. On the other hand if L. M. had continued to excrete bacilli, the excretion by normal carrier being not necessarily as transient as it was in his case, we should have left an unsuspected carrier (S. M.) who might have been the source of infection for subsequent cases.

Although a positive Widal reaction may be absent in chronic carriers, our results indicate that even if a farm helper is found to be excreting typhoid bacilli, any other individual giving a partial or positive Widal reaction should be held under suspicion. At least, fecal examinations should be made over a period of time for excretion even with chronic carriers is intermittent; negative examinations are not infrequent and may extend over months or even for a year.

SUMMARY

The presence on a farm at the same time of a normal carrier and of a chronic carrier, temporarily fecal negative, is a possible source of error in tracing the source of infection of a milk-borne epidemic of typhoid fever.

A COMPARATIVE STUDY OF MILK PLATES BY FOUR NEW YORK LABORATORIES.*

A PRELIMINARY REPORT.

HAZEL M. HATFIELD.

Four of the New York laboratories interested in the bacteriological examination of milk, came together recently for the purpose of determining how much of the variation which occurs in the results when different laboratories attempt to do duplicate work, is due directly to the technical procedure. The object was to gain information regarding the practical application of the milk test by the plate method as performed today by the commercial laboratories and to compare these results with those obtained under identical conditions by the Milk Laboratory of the New York City Health Department.

To determine this point, a set of samples was delivered at the Health Department Laboratory by a collector not connected with the department. They were tested by the plate method in the following manner: Each laboratory represented, used its own sterile glassware, water blanks, agar, etc., exclusively. A definite series of dilutions was agreed upon so that the plates would give results that would be quite comparable. Each sample was shaken by only one worker, who shook the sample in his usual manner. He then removed a portion and immediately passed it to the next worker, who likewise removed a portion (but did not shake). He passed the sample on again until each one had taken what he required. The next sample was shaken by a different worker and so on in turn. From this point on, the work was done according to the individual preference of each worker and, as nearly as possible, in conformity with the routine procedure of the laboratory each represented. The plates were all packed away in one incubator and incubated for 48 hours at 37° C. They were all removed at the same time and counted by the person who made them. Every plate was counted even though some would have been discarded under ordinary circumstances as too poor to be accurate. In such cases the poor plates were indicated.

In general, each plate should have shown one-tenth as many colonies as the plate of the next preceding dilution for the reason that in every case the dilutions stepped up one cipher at a time.

The accompanying tables show how the different laboratories fared in this respect and also how they compare with each other.

It is also of interest to note that samples 4, 5, 6 and 7 were quadruplicates, having been taken from the same can after the milk was well stirred; also that No. 16 was a compound sample consisting of one-half No. 14 and one-half No. 15. No. 8 and No. 9 were taken from the same bottle; samples No. 10 and No. 11 were taken from one bottle.

In addition to these results, a very interesting fact developed. When we came to compare our methods in detail, we found that no two laboratories did their work exactly alike; in fact, there was no point on which all were agreed.

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* Published in Am. Jour. Pub. Health, Dec., 1918, 8, 12, p. 883.

TABLE

	Number of Sample.	Dilution.	Plate Counts.				Remarks.
			H. D.	Lab. A.	Lab. B.	Lab. C.	
Raw Milk	1	1:1000	550 est.	512 est.	320 est.	826	Shaken by Lab. A.
		1:10000	110	87	165	124	
		1:100000	7 est spreader	13	21	44	
	2	1:1000	2,700 est.	688 est.	1,100 est.	1,056 est.	Shaken by Health Dept.
		1:10000	326 est. ½	29	370	488	
		1:100000	3	6	120	133	
	3	1:1000	3,279 est.	2,250 est.	980 est.	Plate not made	Shaken by Lab. C.
		1:10000	424 est. ½	268	275	412	
		1:100000	46	18	67	28	
	4	1:1000	1,120 est.	384 est.	1,440 est.	1,600 est.	Shaken by Lab. B.
		1:10000	100 est.	81	190	406	
		1:100000	7	10	48	144	
	5	1:1000	1,000 est. poor plate	848 est.	940 est.	892 est.	Shaken by Lab. A.
		1:10000	120	122	115	282	
		1:100000	9	13	21	34	
	6	1:1000	460 est. ½	480 est. spr.	440	850 est.	Shaken by Health Dept.
		1:10000	64 spr.	76	160	268	
		1:100000	7	11	28	36	
	7	1:1000	680 est. poor plate	1,250 est. spr.	435	896 est.	Shaken by Lab. C.
		1:10000	128 est. ½	160	105	238	
		1:100000	7	23	21	36	

Certified Milk	8	1:10 1:100	420 est. ½ 51	427 42	718 est. 51	313 82	Shaken by Lab. B.
	9	1:10 1:100	315 poor plate 63	339 36	457 est. 71	279 104	Shaken by Lab. A.
	10	1:10 1:100	370 est. ½ 46	338 33	408 est. 56	364 56	Shaken by Health Dept.
	11	1:10 1:100	350 est. ½ 43	295 33	355 est. 43	230 41	Shaken by Lab. C.
	12	1:100 1:1000	92 est. 17	135 13	254 est. 39	245 18	Shaken by Lab. B.
	13	1:100 1:1000	482 est. ½ 50	*	520 est. 51	374 30	Shaken by Lab. A.
	14	1:100 1:1000	412 est. ½ 44	*	504 est. 63	430 70	Shaken by Health Dept.
	15	1:100 1:1000	522 est. ½ 54	381 21	303 47	512 47	Shaken by Lab. C.
	16	1:100 1:1000	480 est. ½ 44	*	230 35	Plate not made 60	Shaken by Lab. B.

* Results omitted because of a gross error in making dilutions.   Est.—Estimated.

The Health Department laboratory follows the present standard methods of the American Public Health Association in every respect:—The agar is made exactly as described in the Standard Methods; we pour 10 cc. in each plate at a temperature of 40° to 42° C. Our water blanks are all alike, the same amount in each one, the bottles of heavy glass, large enough to give a sufficient air space to ensure thorough shaking. These bottles have metal caps. Every dilution, therefore, is made exactly like every other dilution and the larger dilution is invariably reached through the lower dilution. We make such dilutions as will always give us at least one easily countable plate if the milk is not excessively high count for the grade for which it is labelled. Where the grade is not known, at least two plates are made. We test all pasteurized milks in a 1-100 dilution; raw milks and pasteurized creams in a 1-100 and a 1-10,000 dilution; raw creams in a 1-100, 1-10,000 and 1-1,000,000 dilutions. Although we make only one plate on each pasteurized milk, we really do quadruplicate work in each case because all samples are submitted by the inspector in four portions representing either two or four samples from one source. This gives a more comprehensive view of each source than a single sample could, because it shows up irregularities at the dealer's end of the line, also. It must be remembered, also, that no milk is judged on the results of one set of samples. The files give a survey of a milk supply which can easily be adjudged as good, fair or bad without danger of doing an injustice to the dealer.

Laboratory A uses agar which is intended for certified milk, but is not standard for routine milk work. The medium contains 1.2 per cent market agar and 1 per cent. peptone and is cleared with egg. Laboratory B uses standard ingredients in standard amounts. It clears without egg, but employs a short cut in making up the media. Laboratory C clears with egg; the reaction is neutral to phenolphthalein. The ingredients were not stated. All the laboratories except C use an acid reaction of not more than plus 1. Again, the Health Department Laboratory pours 10 cc. of agar in each plate; Laboratory A pours "about 5 or 6 cc."; B pours "about 8 cc." and C pours at least 12 to 15 cc. Laboratory A prefers to pour agar at 45° C., the others at 40° to 42° C. All the laboratories shook the dilution bottles vigorously twenty-five times, but it was noticed that when the worker from Laboratory C used a tube of water in making a dilution he resorted to a rather gentle agitation instead of the usual up-and-down shake, just enough to make the milk and water appear evenly mixed.

In the matter of dilutions, also, there was the widest variation. The procedure of the Health Department Laboratory has already been described. For the pasteurized milks, Laboratories A and B make a 1-100 and a 1-1,000 dilution, while C makes a 1-100, 1-1,000 and 1-10,000 dilution. Moreover Laboratory B sometimes departs from its rule under certain conditions and makes only a 1-10 dilution. In the plating of raw milks it was found that while the Health Department Laboratory makes a 1-100 and a 1-10,000 plate, Laboratory B makes a 1-1,000 for the lower dilution and C makes a 1-100,000 for the highest dilution. As to the manner of making dilutions, in nearly every case the higher dilution is made through the lower dilution, but, again, we found that Laboratory A when testing certified milks, inoculates each blank with separate portions of straight milk. In this case, Laboratory A actually shakes the milk in the higher dilution only fifty times all told (twenty-five times before diluting and twenty-five times afterward), while the other laboratories shake it seventy-five times.

In studying the table, it will be seen that the greatest variations occurred in plating the raw milks both in regard to the comparison of the different plates of the same dilution and in respect to the serial diminution from one dilution to the next.

This series of comparative tests is a small one from which to draw conclusions, but parts of the table give the following indications:

1. That it is perfectly possible to get comparative results that are approximately alike—when the value of the test is taken into account.
2. When the plates are not too highly seeded and are free from spreaders, the various dilutions can be expected to compare quite closely.

The parts of the table where irregularities are apparent make it evident that the factors of variation in the technique should be further studied and standardized.

OBSERVATIONS AND EXPERIMENTS ON DISH-WASHING.*

WALLACE A. MANHEIMER, Assisted by THERESA YBANEZ.

So far as we know there has been no investigation on dishwashing as performed in public restaurants. That dishes are possible vehicles for disease transmission is recognized in the laws against common drinking cups and in the practice of sterilizing dishes used by patients suffering from infectious diseases. Although the latter practice is almost universal and although it is well known that sometimes persons in apparently good health discharge infectious material from the mouth, no attempt has been made to determine to what extent such material may be transmitted to patrons of restaurants using common dishes. It was decided, therefore, to inspect a representative number of restaurants in New York City, and to test out experimentally the efficiency of the methods in use.

Restaurants can be grouped on the basis of dishwashing, into two classes: 1—those using the hand method of dishwashing, comprising by far the larger number, and 2—those using mechanical apparatus.

DISHWASHING BY HAND METHOD.

Twenty-two of the establishments visited used the hand method of dishwashing. These included both cheap and highclass restaurants.

Inspection.—The glasses used at the drinking fountains of the average quick lunch restaurant are not washed at all. Usually, there are a dozen or more glasses on a shelf above the water faucet, which, after use are rinsed off and placed upside down to drain, with no further attempt at cleansing. These glasses are therefore, little better than poorly rinsed common drinking cups.

The practice relative to the cleaning of glasses in confectionery stores is also poor. Glasses after use are rinsed off and used again without drying for serving soda water to a second patron. The spoons are shaken around in water and stood in metal containers from which, although frequently wet, they are served to the next patron.

Although in the restaurants of this group there is considerable variation in the care exercised to produce clean dishes, the process is substantially the same in all places. The dishes are placed in large dish pans containing warm soap water, are rubbed with a dish cloth and then removed to another dish pan containing warm water for the purpose of rinsing off the soap. They are next removed to a draining board and then dried with a towel. In several of the establishments the dishes are not dried with towels, being allowed to dry in the air, the waiter rubbing them with a napkin to remove noticeable spots before placing them on the table. The temperature of the dishwater was, in every case, below 45 deg. C. This is unavoidable because the dishwasher must constantly introduce his hand into the water.

Bacterial Tests.—Bacterial counts were made from samples of dishwater and from moist sterilized swabs rubbed over cups, spoons, forks, etc. As expected, the counts fluctuated greatly. Naturally after washing dishes, which had contained milk, the dish water counts proved to be high. When, however, as frequently happened, the water was changed at the beginning of the inspection the counts, of course, proved to be low. Consequently no conclusion as to the care exercised in washing dishes can be based upon the bacterial counts obtained either from the dishes or the dishwater. The counts obtained, however, are included below for comparison with those obtained from the water of the mechanical dishwasher to be described later. Although no definite conclusion as to the efficiency of the hand method of dishwashing is advanced as a result of study of the bacterial data, we should like to draw attention to the large number of high counts obtained from dishwater and from swabbings of spoons and cups.

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BACTERIAL COUNTS—HAND DISHWASHING.

Dishwater—per c.c. on agar (24 hrs.)

No. of Bacteria.	No. of Places Investigated.
Between 1-100	1
Between 1000-50,000	5
Between 50,000-100,000	1
Between 100,000-300,000	6
Between 300,000-500,000	4
Above 500,000	1
Not taken	4
Swabbings from spoons.	
Between 1-100	8
Between 100-1,000	6
Between 1,000-50,000	3
Above 50,000	3
Not taken	2
Swabbings from rims of cups.	
Between 1-100	4
Between 100-1,000	5
Between 1,000-5,000	11
Over 50,000	2

BACTERIAL TESTS ON ARTIFICIALLY INFECTED DISHES WASHED BY HAND.

Since little information can be obtained from direct examination of dishes and dishwater, series of tests were conducted for the purpose of observing the reduction in the bacterial counts of dishes artificially infected and subsequently washed in a restaurant. Sets of dishes were smeared with bacteria suspended in salt solution, in broth, and in egg albumen. These bacterial suspensions were left to dry on the dishes before the latter were washed. The bacterial suspensions were made up from a salt solution suspension of an agar slant of Bacillus pyocyaneus. Equal quantities of the suspension of Bacillus pyocyaneus were added to tubes containing respectively 10 c.c. of salt solution, 10 c.c. of broth and 10 c.c. of egg albumen. The dishes were smeared with equal quantities of these suspensions, so that, in a measure, the reductions due to washing would be comparable. The dishes after being washed in the kitchen of a clean lunch room were returned to the laboratory. The surfaces were carefully rubbed with sterilized moist swabs and the washings plated out in agar.

While the cleansing of the dishes was, no doubt, unusually thorough, since the object of the experiment was known by the dishwasher, the reduction effected by the hand washing was even much greater than we expected. The counts were as follows:—

DISHES ARTIFICIALLY INFECTED WITH SUSPENSION OF BACILLUS PYOCYANEUS.

TESTED FOR BACTERIAL REDUCTION AFTER HAND WASHING.

Dishes Tested.	Suspension Used.	Total Count Pyocy.
Washing Tests—		
12	Salt Solution	50– 80
12	Broth	100–150
12	Egg Albumen	100–180
Control Tests—		
6	Infected Not washed	500,000–700,000
6	Sterilized Not washed	2

Tests were made on the possible bactericidal effect of the soap powder used, by suspending bacillus pyocyaneus in solutions of the powder prepared similarly to those used in the restaurant. No material reduction in the bacterial counts was effected. The soap, however, had a high detergent value in removing bacteria previously dried on Petri dishes.

The experiment above indicates that careful washing of dishes by the hand method, especially if soap is used, is highly efficient, even though the bacteria had been dried on from the suspensions of salt solution, broth, or egg albumen. It should be remembered, however, that the washing of these dishes was far more carefully carried out than is usually the case.

Although the above experiment would tend to foster confidence in manual dishwashing, our inspections convinced us that this method is highly unsatisfactory. The dishwater is not changed frequently enough, the dishes are insufficiently rinsed, and soiled towels are used for drying. Flies were observed in contact with the empty dishes even after they had been washed; flies were observed also in contact with the dish towels. During the busy lunch hour, in large restaurants, where several hundred persons are served, dishes must be cleaned rapidly for immediate use. Cleanliness is too frequently sacrificed for speed. If hand washing were carefully performed, we believe it possible to obtain satisfactory results. On the other hand, we think it very difficult to enforce care in the washing of dishes by hand.

Mechanical Washers.

Inspection.—Six establishments using mechanical apparatus for dishwashing were inspected. The apparatus for this purpose varies somewhat, but substantially the following description will answer for all. The dishes are packed in wire crates of proper size to be lowered into a vessel containing boiling soap water. At the bottom of the vessel containing the boiling soap water is a fan attached to an electric motor; when the fan revolves, the water is violently agitated and the dishes thereby washed. After exposure to the boiling water for about one minute, the crate is transferred to another vessel containing clean boiling running water. Here the dishes are raised and lowered several times and then transferred to a drying board, where they are stood on edge to dry in the air. The dishes are so hot that hand drying is not necessary and thus considerable manual labor is saved. Furthermore, this operation is very rapid, one man being able to accomplish the work of several, and at the same time getting much better results. The appearance of the dishes is far better after this process than after the hand washing process.

Bacterial Data:—The temperature of water in the dishwasher in all cases but one (where it was 70° C.) was at or slightly under 100° C. By referring back it will be seen that the temperature of the water in the hand washing process never exceeded 45° C. and was indeed usually under 40° C. The bacterial counts of the water from the mechanical washer were consistently low, never exceeding 10 per c.c. The counts obtained from the spoons and the rims of cups ranged below 50. In one case only did the count exceed 500. These figures offer a striking contrast to those obtained from hand washing tests.

Bacterial tests on artificially infected dishes washed by machine.

A series of plates infected in a way similar to those prepared for testing hand washing, were passed through a mechanical washer. The results can be briefly stated: The test organism (B. pyocyaneus) could not be recovered from any of the dishes. When bottles were used in place of dishes, the organisms could be obtained from the bottom of the bottles in greatly diminished numbers in three cases out of twenty. No doubt, the bacteria were protected from the water by air pockets.

Since infected material from the mouth of one person would hardly be transferred to the mouth of another from the bottom of deep receptables (e.g. cups), we believe the occasional recovery of a small percentage of the organisms from the bottom of deep receptacles to be no serious objection to the general application of mechanical apparatus for dishwashing. The knives, forks and spoons tested, were either sterile or gave very low counts.

The time of exposure and the temperature of the water were sufficient to kill all non-spore-bearing organisms.

SUMMARY.

1. Drinking glasses in restaurants and confectionary stores are generally merely rinsed off, and are therefore not properly cleansed.

2. The amount of care exercised to obtain clean glasses varies greatly in different establishments. Dirty dishwater and towels are used in many places and soiled, greasy dishes, spoons, etc., are served to customers. There is generally insufficient protection against flies. Tests of dishes and dishwater frequently gave high bacterial counts.

3. The hand washing of dishes while capable of producing excellent results is too often carelessly performed. Tests of artificially infected dishes, made after the dishes had been washed by hand in a restaurant with soap powder and warm water showed a bacterial reduction exceeding 99 per cent. The solution of soap powder alone was without bactericidal action, the temperature of the water was not sufficiently high to cause bacterial reduction. The above result was therefore due to the detergent action of soap combined with vigorous rubbing in warm water.

4. The washing of dishes in a mechanical dishwashing apparatus offers many advantages over the hand washing method for the following reasons:—

(a) The mechanical washer is a labor-saving device. Not only is the actual washing accomplished more easily and quickly, but no subsequent hand drying is required.

(b) Dishes washed in the mechanical apparatus have a uniformly clean, glistening appearance.

(c) Bacterial counts of cultures made from dish-water and from swabbings of dishes after washing in the mechanical apparatus indicate that except when bacteria are protected by air pockets in the bottom of deep containers, the high temperature of the water used and the time of exposure are sufficient to effect almost complete sterilization of the dishes.

CONCLUSION.*

In view of the carelessness in washing eating utensils in many of the poorer class of restaurants and confectionery stores and in view of the possible transmission of disease from this source, and furthermore, because of the desirability of producing clean and glistening dishes, we recommend an ordinance requiring the subjecting of all eating utensils in public places to a temperature exceeding 80° C. for one minute before permitting the use of such utensils by a second person. Paper cups should be employed as substitutes for drinking glasses unless the glasses can be made correspondingly safe.

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* Since writing this paper a similar conclusion has been reached by Dr. H. N. Cole (Jour. A. M. A., Vol. XLVII, No. 25, p. 1805) as a result of a study of the etiology of a number of cases of extra genital chancres. Doctor Cole Writes: "There should be stringent laws enforced by careful inspection, requiring the placing of all dishes, glassware and silverware in boiling water for the space of five minutes, even if the microscopic dust be not removed. This would effectively kill all organisms and fully protect us."

COMPARISON OF METHODS FOR DISINFECTING SWIMMING POOLS.*

Wallace A. Manheimer.

ULTRA VIOLET LIGHT.

Ultra violet light has been used abroad for the disinfection of drinking water and in Paris for the disinfection of a swimming pool. In this country, where it has recently been introduced, its application to the disinfection of swimming pools is the most modern device adopted for that purpose. In the matter of operation and handling, ultra violet light would be the most satisfactory method of disinfection thus far introduced if certain objections did not outweigh the advantages. These advantages are that in the first place, nothing which could arouse objections from bathers is added to the water, and in the second place it is automatic—neither too much nor too little could be added by a possibly bungling attendant, since after turning on the light, he would need to give no further attention to the apparatus. Accordingly, the determination of its cost of operation, bacterial efficiency, etc., have been investigated with the utmost interest.

Tests at the 57th Street Y. M. C. A.—The capacity of this pool is approximately 75,000 gallons. The water is circulated through the filters every 24 hours and is thereby maintained clear. It is drawn from the bottom of the deep end and returned after filtration to the surface of the shallow end, being there discharged from a pipe about 2 feet above the surface of the pool, the latter measure insuring considerable aeration of the water. At the time of these tests the water had been in the pool for about 6 months.

Samples of water were collected from the pool at the deep end, from a tap on the pipe returning from the filter, and from the pipe discharging into the pool after the water had been exposed to the ultra violet light. From the analyses of these samples it was possible to determine the number of bacteria in the pool, the amount of reduction due to refiltration, and the amount of reduction, if any, due to exposure to the ultra violet light. Similar analyses were made in all tests on ultra violet light.

Series 1. A count of 20 bacteria to the cubic centimeter in water from the pool at 37 C., was reduced by filtration to 5, and by exposure to ultra violet light, to 2. This reduction of 3 bacteria to the cubic centimeter as a result of exposure to the light, in plate counts might readily be attributed to normal experimental error in pouring plates.

Series 2. Here a count of 250 bacteria to the cubic centimeter for the pool sample at incubator temperature fell to 40 during filtration, and rose slightly, to 42, after exposure to the ray. The room-temperature counts showed about the same result—231 bacteria to the cubic centimeter in the pool sample, 32 in the sample from the filter and 53 in the sample after exposure to the light.

Series 3. In this series the results were approximately the same as before. The water in the pool, which had been agitated by scooping dirt from the bottom, contained 150 bacteria to the cubic centimeter at 37 C. and 416 at 20 C. After filtration the counts were 5 and 52, respectively, and after exposure to the ray 15 and 70. Colon bacilli were irregularly present in the 1-c.c. samples.

Tests at the New York Athletic Club.—In order more thoroughly to investigate the value of the machine for disinfection of water, additional series of tests were planned at the New York Athletic Club.

Series 1. Water from the pool gave a count at 37 C. of 345 bacteria to the cubic centimeter, and at 20 C. of 880 bacteria to the cubic centimeter. After refiltration the counts were 250 and 500. After exposure to the ray the counts were 240 and 730.

Series 2. The experiment was repeated as a control before adding a large number of colon bacilli to the water. Again there was no noticeable reduction in the bacterial counts after exposure to the ray.

Series 3. In order to produce test conditions under which results would be more striking, a large number of colon bacilli from a stock culture grown on agar were added to the pool. The data obtained indicate that while the filters were chiefly responsible for the reduction, the ultra violet light did play some part in lowering the counts.

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* Published in Jour. Infect. Dis., 1917, 20, No. 1.

TABLE 1.

BACTERIAL COUNTS IN Y. M. C. A. POOL AFTER INSTALLATION OF VIOLET LIGHT APPARATUS.*

Source of Sample Tested.	1st Series.		2d Series.			3d Series.		
	37 C.	Colon Bacilli per cc.	37 C.	20 C.	Colon Bacilli per cc.	37 C.	20 C.	Colon Bacilli per cc.
From pool.......	20	0	250	231	0	150†	416†	½
From filter.......	5	0	40	32	0	5	52	0
From violet light.	2	½	42	53	1	15	70	0 .

* Water in pool 6 months.

† Pool had been agitated by scooping up dirt from bottom

BACTERIAL COUNTS PER CC. IN THE SAME POOL BEFORE USE OF ULTRA VIOLET LIGHT FOR DISINFECTION.‡

Day of Test.	1st	2d	3d	4th	5th	6th	7th
At 37 C.................	30	700	4000	2500	2600	5000	14000
At 20 C.................	300	6000	44000	150000	150000	170000	31000

‡ Pool emptied every 2 weeks.

The shortness of the time of exposure of the water to the light probably accounts for the rather poor results. That the machine is of some value in purifying the water is evident from a comparison of the bacterial counts taken at the 57th street Y. M. C. A. before the apparatus had been installed with those after the machine was in use (Table 1). The difference between the two groups is striking. Since the use of ultra violet light was the only change in technic, it would appear that this light exerts a steady reducing effect on bacterial pollution.

TABLE 2.

BACTERIAL COUNTS IN N. Y. A. C. POOL AFTER INSTALLATION OF ULTRA VIOLET LIGHT APPARATUS.

Source of Sample Tested.	1st Series.			2d Series.			3d Series.*		
	37 C.	20 C.	Colon Bacilli per cc.	37 C.	20 C.	Colon Bacilli per cc.	37 C.	20 C.	Colon Bacilli per cc.
From pool.......	345	880	0	600	620	0	3780	2400	1000
From filter......	250	500	0	240	470	0	240	470	10
From violet light.	240	730	0	200	450	1	200	450	1

* Colon bacilli added to the pool.

BACTERIAL COUNTS PER CC. IN THE SAME POOL BEFORE THE USE OF ULTRA VIOLET LIGHT FOR DISINFECTION.†

Day of Test	1st	2d	3d	4th	5th	6th	7th	8th
At 37 C.........	150	1000	200	180	200	300	180	500
At 20 C.........	350	10000	6000	1000	230	3000	300	2000

† Pool continuously diluted with warm filtered water, and emptied every 2 weeks.

In the test in the New York Athletic Club the results would point the other way (Table 2). Here, however, the pool when not treated with ultra violet light was continuously diluted with fresh warm water, so that the comparison of bacterial counts before and after, in this instance, is not easily made.

In view of the facts thus far advanced it would seem desirable to increase the time of exposure of the water to the light.

"LECTROCIDE."

"Lectrocide" is a chemical manufactured through electrolysis of brine water (sea water). It is a greenish chemical with a chlorin odor, altho its main ingredient is sodium hypochlorite.

Two methods of applying this chemical to the swimming pool were tried: (1) The chemical was introduced into the bottom of the deep end through a long pipe. Water from the pool was then poured through so as to drive the chemical below the surface of the pool. Tests lasting for 1 week were conducted while this method of treating the water was in use. (2) During the following week the chemical was introduced into the water through an automatic feed pot attached to the pipe leading from the filter to the pool. The valve on the feed pot was arranged so that the chemical was completely discharged into the water in 24 hours.

The data collected (see coefficient in Table 3) during 15 days of test indicate that the introduction of this chemical into the water in single daily doses is superior to the slow continuous adding of the chemical from a feed pot.

The lectrocide was added so that there was 1 part of chlorin to 1,000,000 parts of water. The bacterial contamination in the swimming pool when treated with lectrocide remained low throughout the whole series of experiments (Table 3, Nos. 1, 2, and 3).

COPPER SULFATE.

Experiments were conducted with copper sulfate similar to those conducted with lectrocide. They were similar also to those tests conducted with chlorin gas and calcium hypochlorite, described later. The object was to secure data upon which comparison of relative efficiencies could be based.

Copper sulfate in concentration of 1 part in 100,000 parts of water by weight was added to the pool daily. It was dissolved in water and added in the same ways as lectrocide; that is, as a single daily dose for the first week and from the mixing pot as a continuous gradual dose during the second week. In direct contrast to the experiment with lectrocide, here the continuous slow adding of chemical was more effective than single daily dosing. Evidently the efficiency of chemical disinfection is dependent not only on the chemical employed, but on the method of adding it to the water.

The efficiency of copper sulfate itself, however, is low when 1 part in 100,000 is used. Bacterial pollution was higher throughout the 2 weeks of test than it was when sodium hypochlorite was substituted. Furthermore, the cost per day for treatment with copper sulfate was $1.04—nearly 8 times that for treatment with "lectrocide." The water was yellowish and its transparency was reduced to such an extent as to draw complaints from the patrons. When the pool was emptied, the tiles were stained, so that scrubbing with oxalic acid was necessary in addition to the ordinary labor of cleaning the pool. In short, as will be seen later, copper sulfate showed the poorest efficiency and the highest cost of application of all chemicals used in pool-disinfection. (See coefficient values in Table 3. The results with chlorin gas, Table 3, No. 6, should not be included in the comparison because of defective apparatus.)

TABLE 3.

COMPARISON OF THE RESULTS OF VARIOUS METHODS OF DISINFECTING SWIMMING POOLS.

BACTERIAL COUNT PER CC.

Day.	1. "Lectrocide" Added in Single Daily Doses.		2. "Lectrocide" Added in Single Daily Doses.		3. "Lectrocide" Added From Feed Pot.		4. Copper Sulfate in Single Daily Doses.	
	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.
1st	350	900	2800	660	18000	16500	3000	28000
2d	10000	8000	5200	8000	5000	13200	40000	84000
3d	10000	7000	2400	2700	400	1400	30000	72000
4th	7500	400	1800	2400	80	2500	30000	42000
5th	200	1000	660	2200	29000	89000	56000	66000
Average daily attendance	498		674		488		500	
Capacity	52000 gal.		52000 gal.		52000 gal.		52000 gal.	
Coefficient*	470		22		1700		4700	

Day.	5. Copper Sulfate From Feed Pot.		6. Anhydrous Chlorin From Needle Valve.		7. Calcium Hypochlorite.		8. Calcium Hypochlorite.	
	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.
1st	750	4000	20	400	1000	200	1400	9200
2d	6800	28000	16400	10000	2400	1600	2200	3400
3d	26000	47000	202600	400000	200	1400	300	900
4th	8000	20000	160	400	3500	3600	6000	18000
5th	15000	28000	4800	36000	80	6000	20	5200
Average daily attendance	514		1261		493		357	
Capacity	52000 gal.		55000 gal.		80000 gal.		75000 gal.	
Coefficient*	2000		2900		360		86	

TABLE 3—Continued.

Day.	9. Calcium Hypochlorite.		10. Calcium Hypochlorite.		11. Calcium Hypochlorite.	
	37 C.	20 C.	37 C.	20 C.	37 C.	20 C.
1st	40	60	250	3600	200	3800
2d	500	1400	1500	1100	150	1400
3d	2	70	20	200	2	180
4th	70	240	40	50	10	10
5th	100	1500	10	1200	10	9000
Average daily attendance	338		200		130	
Capacity	75000 gal.		40000 gal.		40000 gal.	
Coefficient*	86		160		450	

* Note.—Since the bacterial pollution in a pool varies with the attendance and with the volume of the water in the pool, the coefficient was computed according to the following formula:

$$\text{Coefficient} = \frac{\text{Average daily bacterial count}}{\text{Average daily attendance}} \times \frac{\text{Volume of pool}}{1000}$$

The coefficients are merely rough estimates, but they furnish a simple basis of comparison in determining the efficiency of the different methods of treating the pool.

CHLORIN GAS.

Chlorin gas was injected into tap water which was under sufficient pressure to feed into the pipe leading from the filtering plant to the pool.

The analyses conducted for 1 week on this pool were unsatisfactory because of defective apparatus. According to the engineer in charge of baths, the quality of anhydrous chlorin has deteriorated since the war, frequently containing a heavy oil which is carried over when the chlorin tanks are filled. This oil clogs the needle valve of the injector causing interruption in the flow of the gas. For this reason, the data collected do not indicate the efficiency of chlorin gas for pool-disinfection. In tests on another pool,[1] however, chlorin gas proved to be an efficient and easily handled disinfectant.

OZONE.

Series of tests on ozone applied to drinking water were conducted. This chemical has not yet been applied to the disinfection of swimming pools, but its use would have so many obvious advantages that I have undertaken to experiment with it. While there are no figures as yet on cost of operation or experience of its reliability and ease of control as applied to swimming pools, the tests thus far conducted indicate a very high efficiency on the part of ozone as a purifier of water. Within a short time, I hope to report more extensively on this subject.

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[1] Jour. Infect. Dis., 1914, 15, p. 159.

COMPARISON OF EFFICIENCY AND COSTS OF THE VARIOUS METHODS

Copper Sulfate.—Recently Thomas[2] and Levine,[3] contrary to most observers, concluded that copper sulfate is superior to calcium hypochlorite for the disinfection of swimming pools. Levine reports satisfactory results at Iowa State College, using 1 part of $CuSO_4$ to 1,000,000 parts of water.

The results set forth in this paper show that even when 1 part of $CuSO_4$ to 100,000 of water was used, the bacterial pollution in the pool exceeded that with all other methods of disinfection.

DeWitt and Sherman[4] showed that while very small dilutions of some chemicals destroyed tubercle bacilli, 5% solution of copper sulfate was necessary, even when the time of exposure was 24 hours. In their more recent paper on the subject they conclude: "In the short time of an ordinary laboratory experiment and with the small amount of material usually employed, copper is unreliable and unsatisfactory both as a bactericide and as a fungicide. One percent fails to kill all organisms within 15 minutes."

Johnson and Copeland[5] got unsatisfactory results with sewage effluent even when using 1 part $CuSO_4$ to 25,000 parts of the effluent.

Clark and Gage,[6] after an elaborate study of the effect of copper sulfate in various dilutions on water from various sources, summarize as follows: "In conclusion, the writers believe that the treatment of water with copper sulphate * * * has little practical value for the following reasons:

"I. The use of any method of sterilization which is not absolutely effective is dangerous in the hands of the general user, tending to induce a feeling of false security and leading to the neglect of ordinary precautions which would otherwise be employed.

"II. Although the removal of B. coli and B. typhosus is occasionally accomplished by dilute solutions of copper sulphate, these organisms may both live for many weeks in water containing copper sulphate in greater dilutions than 1 : 100,000; and in order to be safe dilutions of 1 : 1000 must be used, in which case the water becomes repugnant to the user because of its strongly astringent taste.

"III. In some instances very dilute solutions of copper sulphate [e. g., 1 : 1,000,000 as used by Levine for treating a swimming pool] * * * appear to have a decidedly invigorating effect on bacterial activity, causing rapid multiplication, when the reverse would have been true had the water been allowed to stand the same length of time without any treatment."

The conclusion of Clark and Gage, that high dilutions of copper sulfate are valueless for disinfecting water, agrees with the results which I obtained in the tests on the 23rd Street Baths. Furthermore, as previously stated, copper sulfate costs about $1.04 a day for a 50,000-gallon pool (before the war about 50 cents) when 1 part of $CuSO_4$ is added daily to 100,000 parts of water. The water takes on a yellowish appearance and becomes less transparent. The tiles lining the pool are somewhat discolored.

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[2] Jour. Ind. and Eng. Chem., 1915, 7, p. 476.
[3] Jour. Infect. Dis., 1916, 18, p. 293.
[4] Ibid., 1914, 15, p. 245; 1916, 18, p. 368.
[5] Ibid., 1905, Suppl. 1, p. 327.
[6] Ibid., 1906, Suppl. 2, p. 175.

*Anhydrous Chlorin.**—This is the cheapest of the chemicals to use, costing about 0.5 of a cent per day for a 50,000-gallon pool. When it is free from oil, it is easily controlled and very satisfactory to operate. As stated, stoppage of the needle valve and consequent interruption of the flow of gas make the determination of its efficiency impossible in these tests. In tests on another pool, however, it proved highly satisfactory.[1]

*"Lectrocide."**—This costs now 18 cents a day (before the war about 9 cents) for a 50,000-gallon pool. It is easily handled, causes no objectionable odor, and has a high coefficient of disinfection for swimming-pool water.

*Chlorid of Lime, Calcium Hypochlorite.**—This is a very cheap disinfectant and one showing by comparative test a high grade of efficiency. It costs about 8 mills per day for a 50,000-gallon pool. It is more difficult to handle than any of the foregoing chemicals because of its unpleasant odor when improperly added to the pool and because of the stoppage of the filters if the lime sludge is thrown into the filtering circuit. These objections can be easily overcome if the following procedure is adhered to:

> Mix the chemical with water thoroughly in a large pail, stirring with a stick so that the active material is brought into solution. Do this in the open air, or in some place remote from the room containing the pool, since, when the chemical is mixed in the room, chlorin gas escapes into the air, causing objection on the part of the bathers to the chemical disinfection.
>
> After the chemical has been thoroughly mixed with water, filter it through cheese cloth into another container, so that the lime sludge can be discarded.
>
> Introduce the chemical into the water at the deep end of the pool through a large pipe extending nearly to the bottom of the pool. A funnel may be needed to pour the chemical in. Do not withdraw the pipe until after pouring some fresh water through it. This method obviates the necessity of pouring the chemical on the surface of the water. The presence of the chemical, either in solution or as a powder on the surface of the water, causes unpleasant odors in the air. Another and simpler method is to drop the pail containing the chemical forcefully into the water. In this way very little of the chemical remains on the surface. With this method and care to prevent odors of the chlorid of lime from escaping into the air, more than a dozen sanctioned Mikveh[7] baths in New York City were treated without complaint from the patrons.

CONCLUSION

The value of ultra violet light as a disinfectant in swimming pools has not yet been definitely determined. (a) Swimming pools equipped with ultra-violet-light apparatus showed lower bacterial pollution during its use, than before its use. (b) A somewhat longer exposure of the water to the light would seem desirable; in most instances bacterial reduction was not observed after the water had passed through the ultra-violet-light apparatus.

In regard to the method of adding chemicals to the water, it appears that the value of the slow continuous addition or of the single daily dosing varies with the chemical used. Copper sulfate gave the better results when added gradually and continuously, while sodium hypochlorite gave the better results with single daily dosing.

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* Chlorine gas and the hypochlorites were added to the water so that there was 1 part of available chlorin to 1,000,000 parts of water.

[7] Survey, 1914, 34, p. 482.

The relative efficiency of chemicals for water disinfection may be expressed as follows:

(a) Calcium hypochlorite,—high efficiency, low cost, not much care necessary in handling.

(b) Chlorin gas,—efficiency high, cost very low, easily handled.

(c) "Lectrocide,"—cost 10 times as much as for hypochlorite, high efficiency, very easily handled.

(d) Copper culfate,—cost high, efficiency low, stains tiles, causes reduction in transparency of water, easily handled.

Final decision on a standard method for pool disinfection has not yet been reached, and can not be until after ozone and other methods still under investigation, have been fully tested. In the 9 pools examined, refiltration was practiced in all cases, a procedure which should be standard in all indoor pools.

The writer desires to express his gratitude to Col. Hines for permitting extensive tests in the public baths; to Mr. Eble, engineer in charge of baths, for very material aid in the collection of data; to Dr. Hammond, president of the New York Athletic club, for permitting a series of tests in the pool of the New York Athletic club, and to Mr. Case of the R. U. V. Co. for setting up an ultra-violet-light machine in the laboratory for testing purposes.

THE SANITARY CONDITION OF MIKVEHS AND TURKISH BATHS.*¶

WALLACE A. MANHEIMER

Mikvehs, strictly defined, are Jewish ritual baths. The term "mikveh," however, as commonly used by the Jewish people includes baths of similar type, though not always of a purely ritual nature. Accordingly, mikvehs can best be classified as (1) sanctioned mikvehs (true mikvehs, sanctioned by the Rabbis) or Jewish ritual baths for women and (2) unsanctioned mikvehs divisible into (*a*) common mikvehs or plunge baths operated at elevated temperatures and used as cleansing baths and (*b*) cold plunges operated in conjunction with Turkish sweat rooms.

The first study of the sanitary conditions of mikvehs was that of Mr. H. F. J. Porter,† who reported, in 1912, on their polluted condition. Mr. Porter urged the writer to investigate these baths more thoroughly and as a result, preliminary studies were published‡ in 1914 and 1915. In 1914 the New York City Department of Health invited the Jewish Community (Kehillah) to cooperate in the formulation of plans to effect the sanitation of the mikvehs, the writer being asked to act as consultant. Plans were drawn up which met with the approval of both the Department of Health and the Board of Authoritative Rabbis of the Jewish Community. In order to carry out the details of the plans, the Jewish Community formed an organization of the mikveh owners, and the dues from the members of this association were used to pay an inspector whose duty it was to enforce the sanitary and religious regulations.

The members of the "Mikveh Owners' Association" as it was called, were put to considerable expense and trouble in carrying out the sanitary regulations, but it was felt that the publicity which would be forthcoming through the auspices of the Jewish Community would so arouse public sentiment in favor of the clean mikvehs, that both the expense and trouble would be justified. Furthermore, the Department of Health contemplated a thorough inspection of mikvehs. Immunity from these inspections would be enjoyed by the members of the Mikveh Owners' Association. The publicity, however, was of a meager nature and the inspections of the Department of Health were interrupted through unavoidable causes. The mikveh owners who were members of the Association obtained scant, if any benefits from their membership and accordingly ceased to pay the quarterly dues. The inspector had to be discharged and in due course the society disbanded.

I. *The Sanctioned Mikveh.*—The Sanctioned Mikveh, as already stated, is the only true mikveh, its use being a necessary part of the Jewish ritual. Stringent Talmudic laws govern the use of the ritual plunge, which if carried out in letter and spirit would make sanitary inspection and control of these baths entirely unnecessary. The ritual dip is required of women at all periods within seven days after menstruation, prior to certain holidays, before marriage, etc. Men are not required by religious law to take the ritual dip, although certain sects of Jewish people have acquired the custom of using the mikveh as a semi-religious practice.

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* Published in Med. Rec., 1918, 93, p. 189.

¶ The details of the inspections were carried out by Mr. David Lehman and Mrs. Theresa Ybanez.

† The Survey, July 27, 1912.

‡ The Survey, April 18, 1914, and Aug. 28, 1915; Jour. Inf. Dis., Vol. 15, No. 1, p. 159.

Inspections and Bacteriological Analyses.—There is in each establishment usually one plunge of about 200 cubic feet capacity, containing water maintained at somewhat higher than body temperature. The daily attendance is small, rarely exceeding 30 and usually 15, The cost of baths in these mikvehs varies between fifteen and fifty cents, the majority being low priced. While the baths are usually reserved for the exclusive use of women, some permit the use of them by men at certain times of the day. The women following, therefore, bathe in the same water previously used by the men.

A mikveh to be sanctioned must be under the supervision of a Rabbi, and the bath taken in it must be taken with due regard to the numerous religious tenets governing the ritual dip. Most important among the religious requirements are those which demand scrupulous cleanliness of the body before ablution in the mikveh is permitted.

BACTERIAL DATA FROM MIKVEH CLASS I.

BACTERIA IN 1 C.C.

	Agar 37° C.	Gelatin. 20° C.	Coli in Bile.	Temp. of Water ° C.	Approximate Attendance.
1	79000	190000	100	36	10
2	1900	8500	1	35	5
3	200	12700	0	42	10
4	2100	6000	0	33	15
5	101600	110000	1	38	16
6	241000	31800	100	30	35
7	60000	3700	10	37	15
8	320	650	1	35	1
9	2700	1400	100	32	..
10	30240	16000	10	35	25
11	945	21200	10	34	5
12	260	300	0	34	5
13	1400	4700	1	35	8
14	158	1800	10	38	8

On the whole the premises of this type of bath are in fairly good sanitary condition. The bacterial data collected from analyses of the water, however, indicate quite clearly that more frequent renewal and periodical disinfection are necessary.

Accordingly all the baths were treated with various concentrations of calcium hypochlorite for the purpose of ascertaining the most efficient quantity of chemical to use. One ounce per 900 gallons proved to be satisfactory under the conditions of application which obtained. As a routine the sanctioned baths (those that were members of the "Mikveh Owners Association") were periodically disinfected and daily renewed with clean water.

Conclusion.—Concerning the sanitation of the sanctioned mikveh, a careful regard for the management of the water, including its daily renewal (or more frequent renewal if used by more than 20 persons daily) and its periodical disinfection,* together with the providing of clean towels, liquid soap, individual drinking cups, sanitary control of premises, etc., should prove adequate to effect suitable sanitary conditions.

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* The disinfection of the water of these baths by the use of an ozone-generating machine is contemplated and the details of the experiment will soon be arranged.

II. *Common Mikvehs.*—The common mikveh consists of a plunge bath, the water of which is maintained at an elevated temperature. This type of bath is frequented for the same reasons as Turkish baths, the mikveh plunge being used as a common cleansing bath by a large number of people.

BACTERIAL DATA FROM MIKVEHS CLASS II.

BATHS FOR MALES.

No.	Count at 37° C. per c.c.	Count at 20° C. per c.c.	Coli per c.c.	Temp. of Water ° C.	Attendance at Time of Inspection.
15	1300	1250	1	50	12
16	10000	13000	1	45	12
17	80000	4700	10	50	20
18	112000	11000	50	40	15
19	3750	8000	50	30	1
20	42000	66000	50	55	150
21	2800	3200	50	35	80
22	3537	2000	50	50	80
23	144000	27000	50	50	60
24	4320000	......	50	50	60
25	37500	11800	50	23	0
26	20000	19000	50	40	2
27	200000	3800	50	45	7

BATHS FOR FEMALES.

No.	Count at 37° C. per c.c.	Count at 20° C. per c.c.	Coli per c.c.	Temp. of Water ° C.	Attendance at Time of Inspection.
28	1800	3200	100	30	10
29	33000	1600	1	35	0
30	27000	11500	1	35	1
31	2000	1500	1	..	..
32	26000	2300	1	40	12
33	11000	9500	1	55	1
34	11300	9500	1	23	0
35	11500	800	1	45	0
36	3600	2600	1	43	0
37	60000	60000	50	35	1
38	6000	77	1	45	0
39	135000	220000	50	33	0
40	31500	43000	50	25	3
41	23000	1000	1	35	4
42	1600	5400	0	40	0
43	1600	800	10	30	10
44	320	250	50	28	5

Inspections and Bacteriological Data.—These mikvehs are located, usually, in tenement houses, where they occupy the ground floors and cellars. Some, however, are located in loft buildings, in yards of tenement buildings, and a few in stables. A separate coal furnace for hot water is invariably on the premises to supply hot water for the mikvehs, tub baths, and shower baths, if present. Coal, wood, straw, litter, and debris of all kinds have been observed about the furnaces, which constitute a grave fire hazard, especially when it is recalled that most of these mikvehs are in tenement houses, accommodating, at times, as many as twenty large families. Concerning the fire hazard for those using the mikveh, conditions are very bad. The approach to the mikveh is frequently by means of a narrow wooden staircase leading down to the cellar, where the bath is located. Destruction of the stairs would cut off the only means of egress for the bathers. Occasionally the public press has reported fires in these establishments which have fortunately terminated, up to now, without fatality. Nevertheless, the fire hazard in these baths, as well as in those described in Section III, is of grave character and worthy of drastic control.

Reliable data on the number of individuals who use the baths cannot readily be obtained; observation, however, showed that large numbers frequent them. Indeed, as many as 300 people have been observed to bathe in a plunge containing a like number of cubic feet of water! The presence in the immediate vicinity of free municipal baths has operated, through salutary competition, to reduce the profits derived from mikvehs, and some of the mikvehs have suspended operation entirely. Since the majority of these baths are not of a purely ritual nature, such competition should be encouraged and the free bathing facilities for the people in those communities greatly expanded.

In most instances the pools consist of tile-lined tanks sunk in the ground and are about 5 feet deep by 6 feet square. Few mikvehs contain over 300 cubic feet of water, the majority containing approximately 175 cubic feet. Most of the establishments have more than one of these plunges, which they operate at different temperatures, the bathers first immersing themselves in the water of that containing the lowest temperature and finally going to the one having the highest. The temperature of the water varies considerably. The highest temperature observed was 55°C.* Most, however, are operated at between 40° C. and 45° C., although numerous instances of water at 50° C. were observed. The water is changed at the end of a day's use, if its appearance absolutely demands it, but is never changed during the day in spite of its being used for common bathing by a large number of people. It was not possible to collect samples of water always at the time of maximum pollution, but the table indicates sufficiently polluted water.

Common combs and hair brushes, cake soap, and common bathtub brushes are usually supplied. Dressing-room compartments are of wood and not vermin-proof. In some of the establishments rows of couches are supplied, as in the mikvehs of Class III (see Section III). In most of these places only bathtubs are supplied for those who desire to take a preliminary cleansing bath, but these tubs are not much used because of an extra charge of 5 cents. Showers are rare and when present have been observed in several instances to empty into the plunges. Bathtubs when used are not carefully cleaned for the next patron and are frequently left to stand for considerable lengths of time full of filthy water, until the attendant has time to empty them. The chains on the stoppers are frequently broken and for this reason the attendant must thrust his arm into the filthy water and remove the stopper. A superficial cleaning of the tub ensues to put it in readiness for the next patron. Poorly laundered towels are frequently supplied, and at times roller-towels for common use provided.

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* The hot water from these baths is frequently added by a pipe emptying onto the surface of the water. This accounts for the high temperature obtained. A few temperatures at the bottom were taken and they registered from 5° to 10° C. less.

From the hygienic standpoint these very hot baths are just as devitalizing as the Turkish baths (see Section IV) and are not recommended by leading hydrotherapists. Taking into consideration the filthy condition of the water, the insanitary conditions of the premises, the fire hazard, and the devitalizing influences of the baths, I feel justified in concluding:

1. That the shower baths, as far as possible, replace the common bathtub, which, if continued to be used, however, must be cleaned adequately after each bath.
2. That increased bathing facilities be provided in the vicinities where these mikvehs exist. That shower bath facilities be provided in the basements of settlement houses, public schools, and other public buildings.
3. That these mikvehs, if allowed to continue business, be carefully supervised as to their sanitary condition.
4. That this type of plunge bath be abolished through the passing of a law preventing the taking of a cleansing bath in a plunge used by more than one person.

III. *Mikvehs with Turkish Sweat Rooms.*—These are border-line mikvehs, partaking of the character of both mikvehs and Turkish baths. They are primarily poor grade Turkish baths provided with a cold mikveh plunge, and without the services of masseurs. In the common mikveh (Class II) sweating results from immersion in the hot water of the plunge, whereas in these establishments "sweat rooms" are provided, which are heated with steam and hot air.

Inspections and Bacteriological Data.—The individuals who use these baths disrobe in a large room and place their clothing either on benches or in small wooden lockers. These lockers are not ventilated and are not vermin-proof. The bathers then proceed to the sweat room, where they remain, seated on long wooden benches until they have sweated to their satisfaction, after which they take a brief dip in the cold water of the plunge. Some elect to omit the dip in the mikveh, taking a cleansing bath instead.

The plunges in these establishments are not used extensively and do not represent so grave a sanitary menace as those described in Class II. In regard to the sanitary condition of the premises, however, these baths present an even poorer picture than those already described.

In particular the sleeping accommodations supplied to patrons deserve mention. The couches are placed close together, usually in poorly ventilated rooms* and in large numbers. Sheets, pillow-cases, blankets and quilts are laundered infrequently and provided for common use. Bed linen in positively filthy condition was observed on the couches.

The bacterial data (see table) from the water of these baths show no striking contrast with those obtained from the previous group of mikvehs. The length of time the water is permitted to remain in the pool probably explains the rather high counts. The water, however, from general appearance seems to be in much better condition, especially since it does not contain the detritus from the body which literally covers the surface of the water in the common mikveh (Class II).

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* As instances of poor ventilation we cite two clippings from a New York newspaper:

"Six men were overcome by coal gas in a Turkish bath establishment. The coal gas was generated by a furnace used for supplying steam for the sweat room."—*The Sun,* Jan. 2, 1917.

"One young man was killed and five persons overcome by coal gas from the furnace in a Turkish bath."—*The Sun,* Jan. 3, 1917.

In one particular only, namely, the appearance of the water of the plunges, do these baths surpass those of Class II; in all other details of sanitary condition they are on a par with them.

MIKVEHS WITH TURKISH SWEAT ROOMS III.

MALE.

No.	37° C.	20° C	Coli in Bile.	Temp. of Water.*	Approximate Attendance.†
45	160000	360000	50	cold	30
46	7200	22000	50	cold	30
47	2000	1500	1	cold	50
48	27000	14000	50	cold	50
49	40000	180000	100	cold	100
50	3100	17500	100	cold	100
51	2300	2000	100	cold	30
52	6000	1300	10	cold	10
53	54000	50000	1000	cold	0
54	1000	4400	1	cold	0
55	1000	1200	10	cold	12

* Where the water was unheated, *i.e.*, cold, the temperature was not taken.
† The men attending the bath do not always use the pool.

FEMALE.

No.	37° C.	20° C.	Coli in Bile.	Temp. of Water ° C.	Approximate Attendance.*
56	48000	21000	1	30	0
57	30000	8000	50	34	8
58	76000	170000	1	30	0
59	80000	510	1	40	6
60	3000	7000	1	25	10
61	240	340	30	30	2
62	88000	11000	1	30	12

* The women attending the baths, always use the pool.

Conclusions.—1. The plunges in mikvehs with Turkish sweat rooms should be operated in precisely the same way as swimming pools. The water in them should conform with the bacterial standard for swimming pool water and should receive periodical disinfection in a manner similar to that recommended for sanctioned mikvehs (Section I). Preliminary inspection of bathers and a preliminary cleansing bath should be required.

2. Adequate supervision of the premises should be maintained. Proper ventilation should be secured. Individual bed linen, towels, brushes, combs, etc., should be provided.

3. Elimination of fire hazard for both the bathers and the dwellers in the tenement should be secured.

IV. *Turkish Baths.*—Turkish bath establishments present a far better appearance than do the mikvehs with Turkish baths, although the ones inspected ranged from rather poor class places to those having the finest appurtenances and up-to-date conveniences. Concerning their hygienic features leading balneologists condemn them.

From the sanitary standpoint, fortunately, their condition is fairly good, only certain elements of their procedure requiring correction.

Inspections and Bacteriological Data.—The swimming pools operated in conjunction with the Turkish baths were examined in the winter and were at that time in fairly good condition (see table). In the summer, however, they are used more extensively, and the conditions would probably not be so good.

SWIMMING POOLS IN TURKISH BATHS.

No.	37° C.	20° C.	Coli in Bile.	Temp. of Water.	Approximate Attendance.*
63	600	2200	1	cold	90
64	210	700	0	cold	30
65	2400	3500	1	cold	75
66	21000	130000	0	cold	70
67	4000	3800	0	cold	10
68	850	1100	0	cold	80
69	7600	12000	1	cold	125
70	102	10000	1	cold	65
71	380	4000	1	cold	75
72	5	18	0	cold	50
73	104	750	0	cold	35

* Attendance here refers to the number of people using the Turkish Bath. There was no way of telling how many used the plunge.

Concerning the sanitary condition of the premises and bathrooms, our reports were generally favorable, clean bedding, bed linen, and properly laundered towels were supplied. Only in a few particulars could objections be raised, as, for example, use of common drinking cups for supplying ice water to the patrons in the sweat rooms, the use by the masseurs of common brushes for washing the patrons, the ungloved and possibly contaminated hand of the masseurs, and the poorly cleaned marble slabs upon which the patron lies while being washed. In addition to these details it should be pointed out that the baths are used during the day by women and during the night by men, and the sleeping quarters get such continuous use as to preclude proper airing of the bedding.

Conclusions.—1. The common drinking cup provided in the sweat rooms should be abolished and replaced by sanitary drinking cups.

2. The common scrub brushes used by the masseurs should be replaced by individual brushes or hempen wads that can be sterilized after each separate use.

3. Marble slabs should be thoroughly cleaned and hosed off before use by a second patron.

4. The masseur should be required to wear gloves for his own as well as the patron's protection and these gloves should be sterilized after individual use.

5. Turkish baths are harmful from the hygienic standpoint, and their use should be discouraged.

STANDARDS FOR SWIMMING POOL LEGISLATION.*

Wallace A. Manheimer

The writer has been consulted a number of times by the health authorities of various communities concerning the requirements of public health ordinances relating to swimming pools and bathing beaches. The widespread interest which has been shown in the proper safeguarding of swimming pools has prompted the brief detailing of the standards up to which the laws enacted should measure. While it is recognized that the requirements of different localities may make the modification of the standards below suggested a necessity, nevertheless, in the main, the problem of pool sanitation is the same in all communities.

Swimming pools may be classified as indoor and outdoor pools, fresh and salt water pools, wading pools, and mikveh baths. The regulations concerning mikvehs have been published in a previous issue. For details concerning outdoor bathing beaches, consult the laws of the State and City of New York.

The regulations concerning pools can be conveniently divided as follows: (1) Regulations concerning the management of the water; (2) regulations concerning the construction of pools, including the reduction of fire hazard and the provision of safety devices; (3) sanitary control of premises and bathers. No attempt is here made to set forth the regulations, merely standards are suggested.

A. Standards Concerning the Management of the Water.

1. The bacterial contents of the water supplied to a swimming pool must conform with that of drinking water, or, if polluted, must be disinfected before use.

2. The water in the swimming pool during use must be continuously diluted by adding fresh water, or, by re-using the water from the swimming pool after filtration. (Note: It is preferable to keep re-using the pool water by a process of circulating it through a filter—refiltration. This will conserve both the water and the fuel for heating.)

3. The water must be maintained clear at all times to an extent at least sufficient to see a submerged person in any part of the pool. (Note: The manner of accomplishing this should be left to the proprietor of the establishment; however, the most satisfactory method so far introduced is by the continuous circulation of the water through a filter—refiltration.)

4. The water must be either periodically and regularly or continuously disinfected, unless emptied daily. The bacterial count must not exceed 10 colon bacilli per cubic centimeter. (Note: No upper limit for total bacterial count should be set until further experience has been accumulated on pool sanitation. The frequent emptying of the pool should not be compelled or encouraged because disinfection and refiltration give as good results as frequent change of water and because the frequent change of water causes needless waste of water, fuel, power for pumping, etc.)

5. The method of disinfection must be satisfactory to the Department of Health. (Note: No method should be prescribed in view of the advances constantly being made on this phase of pool sanitation; chlorine and

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* Published in Med. Rec., 1918, 93, p. 418.

other chemicals, ultraviolet light, and particularly ozone, all have their advantages.)

6. The water from the pool must, when discharged, cause no contamination of any stream used for drinking purposes and should, if possible, be disposed of as sewage water.

B. STANDARDS CONCERNING THE CONSTRUCTION OF POOLS, ETC.

1. The lining of the pool must be white or nearly so, smooth (readily cleansable), and impervious to water. The corners of the pool should be rounded.
2. There must be no obstructions in the water. Stair and stair supports must be of metal or stone.
3. Water from the floor surrounding the pool must not drain back into the pool.
4. The floor must be constructed of material impervious to water, must be adequately drained and composed of material designed to prevent slipping, and, if possible, a poor conductor of heat.
5. There must be a scum gutter on at least two opposite sides of the pool, and preferably completely surrounding the pool, for the purpose of draining off surface dirt and of affording a place for bathers to spit. The scum gutter must drain into the sewer or cesspool and not back into the recirculation system.
6. Construction should be made with due regard to the elimination of fire hazards, taking into account the providing of sufficient exits to accommodate the largest crowd that would be likely to attend exhibitions, etc.
7. The pool should be shallow at one end and deep enough at the other to permit diving with safety, unless constructed as a wading pool for children.
8. Dressing room compartments in indoor pools must be constructed vermin-proof.
9. Bathing establishments should be under permit of the Department of Health to include construction and operation.
10. Adequate shower baths and toilet facilities must be provided; also hot water for showers in indoor pools; toilets must be screened against flies, unless water-flushed and covered by toilet seats.
11. Sanitary drinking fountains wtih pure water must be supplied.

C. SANITARY CONTROL OF PREMISES AND BATHERS, ETC.

1. Adequate light and ventilation must be provided.
2. A temperature of the air during the winter in indoor pools of between 70 deg. and 80 deg. F. must be maintained.
3. No common towels, combs, brushes, or drinking cups may be provided.
4. All towels, suits, etc., provided by the establishment for public use must be sterilized after each separate use.
5. Anti-spit signs must be conspicuously posted. Signs in large letters must be posted in dressing compartments directing all bathers, men and women, to take a preliminary cleansing shower in the nude with warm water and soap and to empty the bladder before going into the pool. All bathers must rinse off the soap before entering the pool room.
6. No diseased or intoxicated person should be permitted to use a swimming pool.

7. Only persons dressed for bathing may enter the pool room.

8. Men and boys must bathe in the nude or be provided with sterilized suits. Suits for men and women must be of fast color and of a lintless material.

9. A life guard must be present at all times. He must be acquainted with the technique of resuscitation of the apparently drowned and have equipment at hand for rescuing and resuscitation. He, or some attendant, must supervise the incoming bathers to enforce the regulation concerning the preliminary bath and to exclude undesirable and diseased persons.

10. The room must be locked during the emptying and refilling of the pool, or other steps be taken, so that bathers will not accidentally dive into an empty pool.

11. The pool when emptied must be well scrubbed.

12. No smoking should be allowed in indoor pools.

13. Cuspidors must be provided in the dressing rooms and pool rooms.

14. If tub baths are supplied the tubs must be disinfected after each separate use.

OZONE DISINFECTION OF SWIMMING POOLS.*

WALLACE A. MANHEIMER.

Since the swimming pool has come to stay, and because of its well recognized value in recreation, it becomes a matter of importance to properly safeguard it so that no injury to the public health will result; indeed if it be properly safeguarded so as to present no source of infectious disease, it represents, it itself no mean instrument of public health. It affords opportunity for salubrious exercise and healthful recreation, both of which are essential to the production and maintenance of the public welfare. In years gone by, when infections traceable on strong evidence, to the pool, were published, the pool was properly frowned upon.

The progress of pool disinfection and control in recent years has overcome the objections which were then advanced and there remains today no further excuse for infections originating from this source.

DISINFECTION.

In the purification of a drinking water supply the engineer disinfects the water once and then safeguards it against subsequent pollution. The very nature of a swimming pool, however, makes this impossible. Here an initial purification is of small value since effete matter is being continuously added, and it becomes necessary therefore to counteract this pollution constantly. Furthermore, the load of organic matter and bacteria in a swimming pool varies with the number and type of people using it, while the load of such substances in drinking water remains fairly constant or fluctuates slowly. It is therefore not a very difficult matter, presupposing proper design to adjust the apparatus so as to destroy the average load of pollution which is in a drinking water supply. In pools this adjustment would involve constant modification if a nice balance is to be struck and no objectionable odors produced through the use of an excess of the chemical reagent.

The addition to the water of chemicals in concentrations greater than necessary to effect initial purification for the purpose of disinfecting all subsequently added pollution unfortunately has not worked out in practice; and because of the astringent condition of the water resulting, all attempts in this direction have been discontinued. In order to overcome as far as possible the continuously added polluted matter the recourse has been to dilution. At first, reliance was placed on the addition of fresh, warm, filtered water, but the cost of this procedure was so high that it had to be abandoned. Furthermore, when a turbid city water supply was used, it was found that to add the dilution water in sufficient quantities to counteract the pollution in the pool the filters had to be operated beyond their capacity, and thus the water became dangerous on account of its opacity, in that a submerged person could not be seen. As a matter of fact several deaths have unnecessarily occurred as a result of such opacity. For the reasons just set forth it was deemed better to refilter the pool water which is already fairly clear, and to rely on disinfection for further purification. Accordingly, I have recommended as a standard procedure the refiltration of the water for the purpose of clarification and the chemical treatment of the water for purification.

Now, the only objections that have been urged by the sensitive to the practices of refiltration and disinfection are on the esthetic side. It is claimed that the water after refiltration and disinfection contains urine, dissolved organic substances and fecal matter. This I believe is not so. The filters if properly operated will remove solid substances, and the process of disinfection if properly carried out can be relied on to bleach the dissolved coloring matter and to reduce any dissolved effete substances to chemical entities far removed from the sources from which they have sprung. Indeed, after a little careful study these sensitive people would feel that not only is the danger from refiltered and disinfected water far less than it is from the average town water supply, but they would agree that the average water supply is likely to contain even more actual waste products although perhaps not so much of their oxidizable derivatives.

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* Published in Mind and Body, Jan., 1918.

If I have carried my point concerning the feasibility and desirability of the practices of refiltration and chemical disinfection there remains merely the selection of the best method of disinfection.

METHODS OF DISINFECTION.

In the preliminary attempts to purify water, strong disinfectant chemicals were used and subsequently neutralized or precipitated. These attempts worked out satisfactorily in the laboratory but were not applicable on a large scale to the pool, especially in the hands of untrained attendants. As a result, it became necessary to select suitable chemicals and use only as much as was required for disinfection. Here again technical ability is needed for the determination of the exact amount of those chemicals usable only in minimum quantities.

Up to now the chemicals used for water purification in pools have been the hypochlorites and copper sulphate. The hypochlorites from all points of view are superior to copper sulphate, but are themselves objectionable on account of the alteration in the taste of the water, and because of their irritating properties.

Although this objection can be somewhat overcome by proper management of the water, we again need the technical knowledge which is not usually obtainable under conditions of practical application.

More recently ultra violet light has come into the field. Ultra violet light, however, is not capable of penetrating turbid water and its efficiency is reduced in water containing coloring matter or colloidal substances. These deficiencies are part of nature and cannot be overcome by man. Moreover, in application the exposure given the water by the average ultra violet light machine is insufficient to produce any marked bacterial reduction.

CONDITIONS TO BE SATISFIED IN DISINFECTING SWIMMING POOLS.

The conditions which any method of disinfecting a swimming pool should fulfill are:

(1) Automatic control, that is, control involving no technical ability on the part of the pool attendant;

(2) Reliability of disinfecting power under all conditions of practical operation;

(3) The adding of no objectionable material to the water;

(4) The bleaching and changing of all dissolved bodies in the water so as to reduce them chemically as far as possible from the sources from which they spring.

I submit, that a satisfactory method of accomplishing all the above conditions is by the addition of ozone to the water, under proper conditions.

OZONE.

Ozone is a powerful oxidizing agent usually designated by the chemical symbcl Oz. It is a highly unstable gas, breaking down when in contact with organic matter and bacteria, liberating oxygen in the active nascent condition. Because of its oxidizing properties it is a bleaching agent and a powerful disinfectant. It is only slightly soluble in water where its presence is not objectionable or noticeable. When it is passed into the water it bubbles through purifying the water, improving its appearance and leaving no trace of itself. Its action can well be compared to ordinary combustion and thus waste matters coming into contact with this gas are effectually destroyed.

Ozone is produced commercially by passing atmospheric oxygen through a field permeated with a silent electrical brush discharge. That the production of ozone can be accomplished on a commercially practicable scale for water purification is proved by the fact that it is used in many cities in Germany, France and Russia. According to Spaulding, there are 49 large ozone plants abroad having a daily water delivery capacity of over 84,000,000 gallons. The plant at Petrograd alone delivers 24,000,000 gallons a day and the plant at Paris over 12,800,000 a day. Although ozone has been successfully applied to the purification of drinking water, so far as I know its obvious application to swimming pool purification has not hitherto been made.

When it is realized that ozone is a powerful oxidizing agent it can be postulated that all that is necessary to effect disinfection is to bring this oxidizing agent into intimate contact with the bacteria. Furthermore, only that amount of ozone need be used which is sufficient to oxidize the organic matter in the water. Accordingly, if even the slightest excess of ozone be present, the water will be sterile. The only proviso to this is intimate contact of the ozone and the water.

Now, ozone is never produced and isolated pure; it is always in mixture with atmospheric air in various concentrations and it is just this complication which at times in the hands of the amateur defeats its application to water purification. In my own experiments I have found that when high concentrations of ozone in air are introduced into water, sterility or high grade purification results; and this result is invariable. There might be one objection to using high concentration of ozone, namely that of cost. When we consider, however, the comparatively small amount of ozone that is needed in pool purification this objection is not a serious one, although it is one which should if possible be overcome. I should mention, however, that when high concentration of ozone is here stated, not more than three parts per million is meant. When this amount is used, sterile water is produced. Naturally if a plant generates its own electrical current the cost of production of this amount of ozone would be small, and where current is bought, the cost would be nominal.

For the purpose of reducing the cost of application and the attendant waste of ozone, in my recent experiments I reduced the quantity of ozone in the air which was pumped through the water. In the apparatus that I used however there was no method of reducing the total volume of air pumped through the water, because a plunger pump was used for synchronously pumping air plus ozone, and water through the mixing tower.

Accordingly, there was the same gas surface contact with a reduction of the percentage of ozone in the gas, and the result was, as should be expected, low grade purification. From this it must be concluded that either a high concentration of ozone in air must be employed for water purification or some method must be devised for breaking up the air pumped through the water into fine parts to insure intimate contact of the bacteria in the water with the dilute ozone present in the air. I am working on this phase of the question and am trying to devise the mechanical means for insuring close contact of water and ozone in high dilutions. Should this be successful the application of ozone will cost very little. On the other hand, if ozone in high concentration in air must be used thoroughly reliable results will be obtained, although the cost of application will be somewhat increased. It may be that a smaller volume of air can be passed through the water without undue loss of efficiency. Should this prove the case, a high percentage of ozone in the air could be used, but the total amount of ozone will be reduced proportionately to the reduction in the volume of air. Whether or not this will produce sufficient surface of contact of the gas to the water to effect disinfection remains to be seen. Should this prove satisfactory the only change necessary in the apparatus would be a control on the amount of air pumped through the ozonator.

While several as yet unsolved problems have been suggested in this paper, I wish to be perfectly clear in my conclusions which are:

CONCLUSIONS:

(1) Ozone efficiently purifies water when used in sufficient concentration and if proper contact of the ozone and water be effected.

(2) The application of ozone to the purification of swimming pools promises to be a distinct advance over all methods so far introduced, because

(a) It is automatic in its control entailing no technical knowledge on the part of the attendant,

(b) It is reliable in its disinfecting power under all conditions of practical application,

(c) Nothing objectionable is added to the water. When added in excess of the amount necessary to oxidize all organic matter, it merely bubbles through the water in which it is only slightly soluble. It can be used therefore without annoyance in an amount far in excess of that necessary to effect purification, a condition which the chemicals used for pool purification have never fulfilled.

(d) It oxidizes all organic substances in the water changing them chemically so that they no longer resemble the substances from which they were derived.

(3) That at present due to the methods employed in emulsifying the water with the ozone it is necessary to use an excess of ozone at the expense of a slight financial loss.

In my next paper* I shall give figures on the electrical current consumption per gram of ozone production with the Sprague Ozonator, which is selected because of its high efficiency. I shall also report on the results of experiments with mechanical mixers and shall endeavor to present an arrangement of apparatus and the details of its installation that will be applicable to the average swimming pool.

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* The experiments so far conducted and those contemplated have been made possible as a result of the cooperation of Mr. Willard A. Kitts, Jr., of the Pitts and Kitts Manufacturing and Supply Co., of New York.

THE APPLICATION OF OZONE TO THE PURIFICATION OF SWIMMING POOLS.*

WALLACE A. MANHEIMER.

Recirculation and disinfection of swimming pool water may now be accepted as standard and mutually interdependent procedures in the sanitary control of plunge baths. Experimental data[1] collected during the past several years have demonstrated beyond doubt the necessity of resorting to refiltration to aid in swimming pool purification unless fresh, warm, well-filtered water is constantly supplied to the pool. In the latter process the waste of water, and of coal in heating it, coupled with the difficulty of removing all suspended matter by an initial filtration are sufficient reasons for adopting refiltration of the pool water as a standard procedure. A clear water is essential not mainly because of esthetic reasons but for the reduction of the hazard of drowning. No method in actual practice measures up to refiltration.

Concerning the methods of disinfection, the writer has already set forth[2] the data and conclusions from a comparative study of all the chemicals hitherto applied to pool water purification. .Ultra-violet light in actual operation was not considered a sufficiently powerful germicide. Copper sulphate was expensive in application and unreliable unless large quantities were employed, at which time the water became disagreeable to swim in. Furthermore, the transparency of the water was impaired and the tile lining of the pool stained, entailing additional labor in cleaning the pool.

Chlorine compounds, calcium hypochlorite, sodium hypochlorite and gaseous chlorine, while effective to a marked degree as disinfectants, required, for satisfactory use in swimming pools, a type of technical control usually not available. A study of the practical application of these chemicals to swimming pool purification has demonstrated in the large majority of pools that either the quantity added was insufficient to effect proper bacterial reduction or that the water had a disagreeable odor and taste due to the presence of an excess of the reagents. It is difficult to strike a nice balance between odor and taste on the one hand and bacterial purity on the other in the case of chemicals usable only in minimum quantities.

In consequence of the disadvantages attending the use of the common chemicals for water purification when applied in swimming pools the writer decided to attempt the application of ozone for this purpose.

The application of ozone to water purification presents no novelty. Vosmaer[3] in his comprehensive treatise gives no less than one hundred and fifty-one references to literature on this subject. According to Spaulding[4] there are forty-nine large ozone plants abroad, having a daily water delivery capacity of over 84,000,000 gallons. The plant in Petrograd alone, delivers 24,000,000 gallons a day and the plant at Paris over 12,000,000 gallons a

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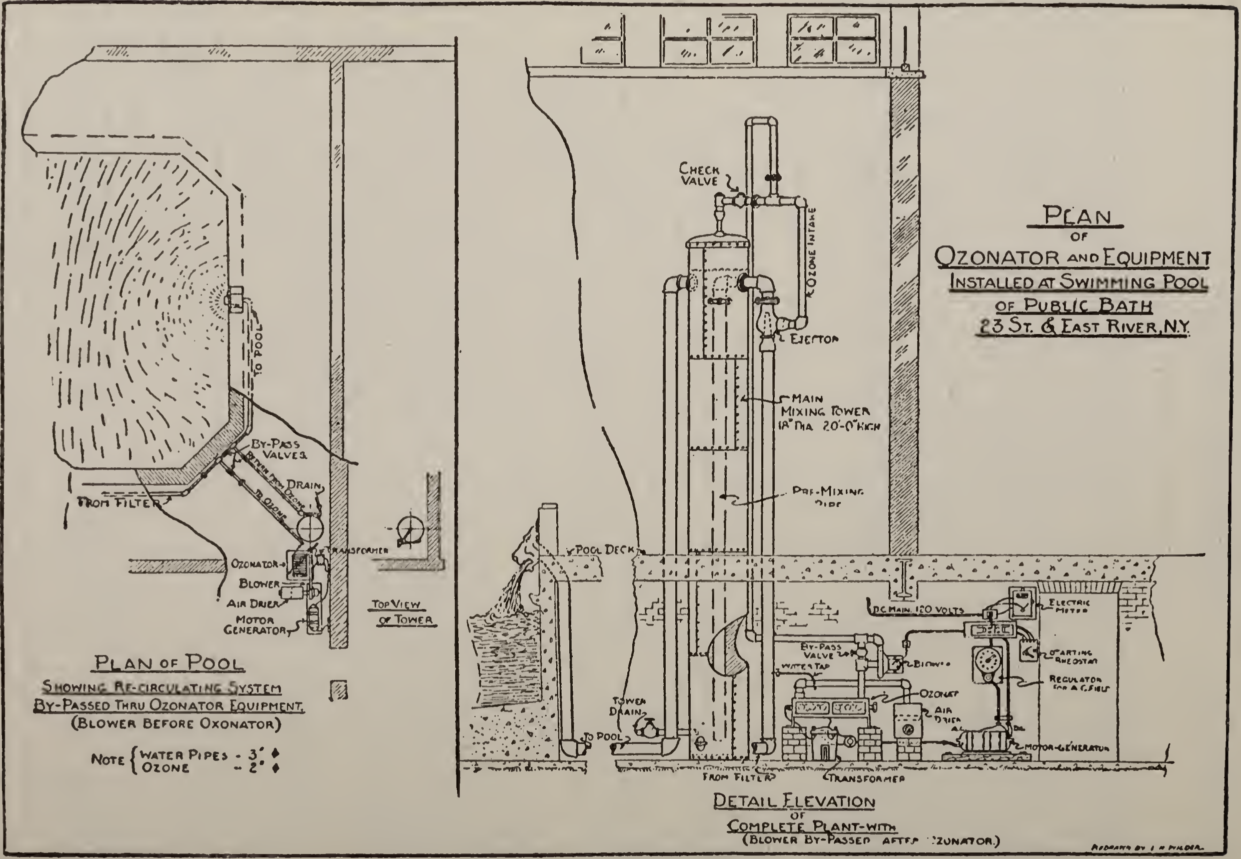
* Published in Public Health Reports (U. S. Public Health Service), 1918, 33, No. 9.

[1] Am. Phy. Ed. Rev., 1912, 17, p. 669; Jour. Inf. Dis., 1914, 15, p. 159; U. S. Pub. Health Rep., Sept. 17, 1915 (reprint No. 299); Jour. Inf. Dis., 1917, 20, p. 1; Jour. Am. Soc. Promote Hyg. & Pub. Baths, 1918, vol. 1, p. 19.

[2] Jour. Inf. Dis., 1917, vol. 20, No. 1, p. 1.

[3] Vosmaer "Ozone" D. Van Nostrand Co., 1916.

[4] Spaulding: "Report on Application of Ozone to Water Purification," N. Y. State Dept. of Health, 1913.



PLAN
OF
OZONATOR AND EQUIPMENT
INSTALLED AT SWIMMING POOL
OF PUBLIC BATH
23 ST. & EAST RIVER, N.Y.

PLAN OF POOL
SHOWING RE-CIRCULATING SYSTEM
BY-PASSED THRU OZONATOR EQUIPMENT.
(BLOWER BEFORE OXONATOR)

DETAIL ELEVATION
OF
COMPLETE PLANT-WITH
(BLOWER BY-PASSED AFTER OZUNATOR.)

day. Rosenau[5] in a summary of this question states: "Ozone is one of the most satisfactory methods of purifying water from a sanitary standpoint. As a germicide it is the most effective of all methods used, except boiling. A well-ozonized water is practically sterile and the organic matter is partially oxidized. It is true that a few resisting spores are not killed, but these are harmless when taken by the mouth."

The writer first applied ozone to the purification of a miniature pool. He concluded from his experiments[6] that ozone efficiently purifies water if proper contact of ozone and water be effected; that the application of ozone to pool water is automatic in its control and reliable in disinfecting pools. Furthermore, it can be added without objectionable result in any excess, since it is not very soluble in water. It oxidizes most of the organic matter in the water and is inexpensive in application.

As a result of these preliminary tests, the writer, in an effort to determine the applicability on a large scale, secured the installation of a large ozone plant at the Twenty-third Street Bath, New York. The following data have been gathered from tests on that installation.

Details of Installation.

The writer soon found out in his preliminary experimental work on ozone[6] that the engineering details connected with the application of ozone on a large scale are of paramount importance. The most important feature in the application of ozone to water is the securing of a thorough mixture of ozonized air and water. No doubt the action of ozone on bacteria and organic matter is exceedingly rapid, as was evidenced by the preliminary work, but if the time of exposure of the water to the ozonized air is short, three parts per million of ozone (which is at least three times the necessary quantity) are required to obtain satisfactory results. A mechanical mixer was designed to insure intimate contact of the gas with water, but this was abandoned for the simpler and surer method recommended by Vosmaer, namely, the use of a tall tower.

It was finally determined to install a 20-foot tower in the Twenty-third Street Bath and to pass the water of the swimming pool into the bottom of this tower after filtration. If this tower is constructed so that the top is on a level with the surface of the pool, there is practically no additional cost in the operation of the recirculation system.*

Two systems of introducing the ozone into the water were installed at the same time so as to ascertain their relative merits. One was the use of a common ejector (see diagram) and the other the use of a small centrifugal air blower. The ozonized air is introduced into the pipe leading down through the center of the tower, where both water and ozone are liberated. The ozone, of course, bubbles through the water quite rapidly, the water (in its slower upward passage) being thus brought continuously into contact with fresh ozone. The ozonized water is then drawn off from the top of the tower and returned to the shallow end of the pool.

It was found when the circulating pump was stopped that water backed down into the ozonator. The air in the pipe which carries both air and water into the bottom of the mixing tower rises and carries the water with it. By a special arrangement† of pipes and valves, the water and ozone from this pipe are returned to the mixing tower (see diagram).

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[5] Preventive Medicine & Hygiene, D. Appleton, 1913, p. 794.

[6] "Ozone Disinfection of Swimming Pools," Journal of the American Assn. for Promoting Hygiene & Public Baths, 1918, Vol. 1, No. 1, p. 19.

* The writer is indebted to Mr. S. Palmer for many practical suggestions and for the accompanying drawings which set forth the details of installation.

BACTERIAL DATA.

The bacterial counts in this pool were so low after continuous operation of the ozone machine, that it was deemed advisable to add a large number of *B. coli* to the water in order to observe the efficiency of ozone on heavily polluted water. Accordingly a mass culture of *B. coli* was emulsified in salt solution and thrown into the pool. In order to secure thorough mixing of the bacteria with the pool water and to keep the pollution high, the circulating pump was shut down between 10 a. m. and 2 p. m., a long-handled brush was used to stir the water, and this, together with the agitation produced by the bathers, resulted in a uniform mix. The pool was then operated as usual and 2 hours later the tests were made.

TEST USING ONE PART OF OZONE PER MILLION OF WATER.

(Note.—All bacterial counts are averages of three or more determinations.)

Using ejector:
- Bacterial count in artificially infected pool, 3,700 per cubic centimeter.
- After filtration and before ozonation, 1,850 per cubic centimeter.
- After ozonation, no growth in 1 cubic centimeter.
- After ozonation, no growth in 3 cubic centimeters.

In addition to plating one cubic centimeter of the water delivered from the ozone tower, three cubic centimeter samples were plated as well. In the majority of cases no growths were obtained.

Using blower:
- Pool water in the artificially infected pool, 3,500 per cubic centimeter.
- After filtration and before ozonation, 1,540 per cubic centimeter.
- After ozonation, no growth in 1 cubic centimeter.
- After ozonation, no growth in 3 cubic centimeters.

The results, using the blower, are identical with the foregoing. We conclude therefore that when one part per million of ozone is used, either with an ejector or with a centrifugal blower (which delivers more air) the pool water artificially contaminated with *B. coli* is sterilized.

TEST USING 0.5 PART OZONE PER MILLION OF WATER.

The foregoing results were so striking that it was determined to cut down the amount of ozone supplied to the pool to ascertain the safety factor allowed when supplying one part of ozone per million of water. For this purpose the output of the ozonator was reduced to one-half by removing three of the six tubes.

Using ejector:
- Count from pool (artificially infected), 2,700 per cubic centimeter.
- After filtration and before ozonation, 1580 per cubic centimeter.
- After ozonation, 1⅓ colonies per cubic centimeter.
- After ozonation, 1 to 3 colonies in 3 cubic centimeter samples.

When using half part of ozone per million of water and introducing the ozone by means of the ejector, the contaminated pool water was delivered in a relatively pure condition. A pool operated in this way, using half the quantity designed, while not delivering entirely sterile water, would be sufficiently pure for all practical purposes.

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† I am indebted to Mr. Willard A. Kitts, Jr., for the ingenious solution of this problem.

Using blower:
Count from pool (artificially infected), 3,020 per cubic centimeter.
After filtration and before ozonation, 1,150 cubic centimeter.
After ozonation, 130 colonies per cubic centimeter.

When the blower was used a larger number of bacteria escaped destruction. This was due, in the writer's opinion, to the larger quantity of air introduced into the ozonator, resulting in a dilution of the ozone, and to the more rapid escape of the gas from the water. A noticeable odor of ozone is observed when the blower is used, indicating that the ozone escapes without a sufficient opportunity of mixing with the water. The conclusion is reached, therefore, that the use of a turbine blower is inferior to that of an ejector, and that a great dilution of ozone in air is a disadvantage.

Cost Data.*

The installation cost in normal times would probably be at the rate of about 3 cents a gallon of capacity of the swimming pool. This cost will naturally vary from time to time with the cost of labor and materials. The electrical current consumption when using alternating current is 2 kilowatts per day, which amounts to from 10 to 14 cents if the cost of current varies between 5 and 7 cents per kilowatt. To this should be added 1 cent a day for the maximum cost of replacing the calcium chloride in the air dryer. The total daily cost of operation with alternating current for 60,000 gallons of water is between 11 and 15 cents. If direct current is supplied, a motor generator must be used, increasing the cost by from 10 to 15 cents a day (2 kilowatts). These figures are based upon local prices of electrical current. In plants where electrical current is generated on the premises, the cost, already very low, will be brought down still more.

The plant when once installed and adjusted is automatic and requires practically no care. The water is bleached, gradually assuming a transparent blue color, enhancing the appearance of the pool and decreasing the hazard from drowning. There is no occasion for renewal of the water, which can be retained for an indefinite period in a pure state.

The cost of water in New York City is at the rate of $1 per 1,000 cubic feet; thus, to fill the pool costs $8 for water alone. To heat the water during the cold season from the temperature in the main to that of the pool requires an average expenditure of 3 tons of coal, costing about $18. The cost of labor for cleaning the pool is about $4.† The total cost is about $30. By using the ozone system the saving in the course of a short time will make up for the cost of the installation, while maintaining a clean and safe pool.

The above operating and installation costs are estimated on the basis of one part of ozone per million parts of water, which under the average conditions that obtain in swimming pools allows 100 per cent safety factor in water sterilization.

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* The writer is indebted to Mr. G. S. Eble, engineer in charge of baths of the borough of Manhattan, for many of the figures on cost.

† In municipal pools, while there would probably be no reduction of the pay roll on account of other duties to which the attendants could be assigned, nevertheless a labor saving of at least $4 is effected. Three attendants are occupied for about two and one-half hours in scrubbing out the pool.

SUMMARY AND CONCLUSION.

1. Refilteration combined with disinfection of swimming-pool water is a standard procedure in the sanitary control of swimming pools.

2. The methods of disinfection hitherto employed are objectionable on one score or another. Ultraviolet light in actual practice has proved ineffective; copper sulphate in low dilutions is unreliable and in high concentration is not only costly, but produces a water disagreeable to swim in; chlorine compounds, while effective as disinfectants, require a technical control usually not available in swimming pools, and when used in excess produce objectionable taste and odors in the water.

3. Ozone when properly applied to the water of a swimming pool effectively purifies the water. When one part of ozone per million parts of water is used, the result is sterile water. When half part ozone per million parts of water is used, a bacterial reduction of 99.8 per cent. results, except when too great an excess of air is introduced with the ozone.

4. A study of the cost of operation of the ozonator has shown that a current consumption of 2 kilowatts per day with the alternating current and of 4 kilowatts per day with direct current, plus 1 cent a day for calcium chloride, represents the total operating cost of a 60,000-gallon pool. This amounts to 11 to 15 cents a day for alternating current (at 5 to 7 cents per kilowatt) and to 21 to 29 cents a day with direct current. The cost of refilling the pool is at least $30. The use of the ozonator decreases the number of times the pool must be emptied to such an extent that the cost of the installation is soon paid for.

5. The application of ozone to the purification of swimming pools is automatic in control, reliable in action, and inexpensive in application. Accordingly, we recommend the consideration of this chemical as a standard procedure in the sanitary control of swimming pools.

The writer desires to acknowledge his appreciation of the assistance which both Mr. Todd, of the New York City Bureau of Public Buildings and Offices, and Mr. W. A. Kitts, Jr., have rendered in making the above experiments possible.

LAUNDRIES AND THE PUBLIC HEALTH.

A Sanitary Study Including Bacteriologic Tests.*

M. C. Schroeder and S. G. Southerland.

The purpose of this study was to ascertain the methods employed in New York City in laundering clothes, with especial inquiry into the efficacy of such methods in destroying pathogenic bacteria and thus preventing the spread of disease. The survey covered the following points:

1. Methods employed in handling clothes in the homes.
2. Methods employed in handling clothes in the homes of laundresses.
3. Methods employed in handling clothes in the hand laundries (white, Chinese).
4. Methods employed in handling clothes in the steam laundries.
5. Series of special tests of goods to note the effect of heat upon bacterial life.
6. A canvass of steam-laundry managers to ascertain their opinions concerning the use of nets in laundries.

Part I.—Hand Laundries.

Laundry Cared for in Homes of Customers—As a rule, clothing washed in homes receives a good deal of care. The white clothes receive a preliminary soaking in soap solutions and a thorough washing and scalding or boiling together with drying in the open-air. The colored clothes are also carefully handled and care is given to the ironing processes of both types of clothes. The danger, therefore, from infected linen under ordinary conditions is negligible.

Laundry Cared for in Homes of Laundresses—The methods employed in this type of washing vary according to the standard set by the individual. There are no rules governing the sanitary conditions in the homes of laundresses and there is no municipal supervision of any kind. The clothes from two or more families are frequently washed in the same water. The drying facilities usually consist of pulleys in the back yards or lines on roofs or lines strung in the kitchen or bedroom. The ironing is usually done with some care, and this tends to eliminate infectious material; but owing to the close quarters in which the laundresses live, there is a possibility of a reinfection of the clean linen, if communicable diseases are present among the members of the laundress's family.

Laundry Cared for in Hand Laundries—Most of the clothes washed in New York City to-day pass through the so-called hand laundries, which number approximately 2,800. The laundries may be roughly divided into three groups:

Group 1. Laundries known as feeders for the steam laundries and maintained by them for receiving, sorting, marking, and distributing only.

Group 2. Laundries operated by private individuals who do some washing and practically all the drying and ironing on the premises.

Group 3. Laundries operated by private individuals doing on the premises practically all the washing and ironing for the patrons. This group comprises all of the Chinese laundries and a few of the smaller laundries.

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* Published in Public Health Reports, U. S. P. H. S., 1917, 32, p. 225.

Group 1. This type of laundry is usually in first-class sanitary shape. The premises are not used for living purposes and the business is conducted with due regard to sanitation.

Group 2. In this group we have data concerning 69 laundries:

The average hand laundry in New York City is simply a collecting and distributing station, where all kinds of clothes from all classes of people are received and handled.

Location—These "hand laundries" are usually located in stores which are partitioned off either by full or dwarf partitions into various rooms used for the reception of clothes, washing and ironing, packing for distribution, eating, cooking and sleeping.

The largest hand laundry in the Borough of Manhattan occupied 1,786 square feet of space, with 174 square feet of window light. It had no communicating living quarters and employed six workers.

The smallest laundry in Manhattan had 125 square feet of floor space with 56 square feet of window light, with one communicating living room. It contained two ironing benches and employed two workers. Drying was done indoors on lines strung below the ceiling.

In one laundry we found 459 square feet of space divided into three rooms by means of dwarf partitions. There were eight persons living in this laundry.

The laundries in Brooklyn on the average had larger and better quarters than those in Manhattan.

Of the 69 laundries controlled by white persons which were visited, 45 or 65.21 per cent. had communicating living rooms. The number of living rooms attached varied from one to three and the number of persons living in these rooms varied from one to nine.

A number of laundries were operated by the members of the family living on the premises, children and adults working alike. In many cases, however, we found that help was hired and these additional persons must be remembered in forming an opinion concerning overcrowding.

In Table I a summary of occupancy is given.

In considering this table we must bear in mind that most of the clothes sent to the steam laundries are returned "wet" to the hand laundries. The presence of large quantities of wet clothes in the process of drying adds materially to the crowded conditions. The dampness arising from the wet garments constitutes another possible injurious influence upon the health of the worker. Taken together, all of these factors result in a large number of instances in a condition of overcrowding which may be considered as dangerous to public health and, taken in conjunction with the fact that work for the general public is done in these places, forms a violation of the State factory law.

Methods Employed in Handling Clothes—A large number of statements have been gathered as to the close contact of soiled clothes and clean linen ready to be sent to the homes of the patrons, and of soiled clothes in contact with the wet clothes returned from the steam laundries. While no well-authenticated direct infection can be traced to these practices, it is not impossible that infection can and does take place.

The marking is done by hand, and it is stated on good authority that infection among workers has taken place from this cause.

Sorting—As a rule the shirts and collars, bed and table linen, are bundled separately and then the rest of the clothes are placed in large netted bags, popularly known as "nets." These nets are classified under three heads according to the articles which they are supposed to contain—white, colored, flannels. In the lower grade establishments the shirts, collars and cuffs and bed and table linen may be slipped into the center of a "net" in order to avoid paying the higher prices for this type of work. In the better-class establishments an effort is made to separate the white from the colored clothes and to place them in appropriate bags, but in the majority of places this care is not exercised and the "net" is not so divided. Consequently, anything from the finest white waists to dark overalls or socks may be found in the same receptacle marked white, colored or flannels as it may happen. These nets are *not opened* during the process of washing.

TABLE 1.

One Room.		Two Rooms.		Three Rooms.		Four Rooms.	
Size of Plant.	Number of Persons.[1]	Size of Plant.	Number of Persons.[1]	Size of Plant.	Number of Persons.[1]	Size of Plant.	Number of Persons.[1]
Sq. ft.		Sq. ft.		Sq. ft.		Sq. ft.	
544	6	175½	2	187	3	225	5
392	6	210	2	212½	2	378	3
360	4	459	8	168¾	1	252	6
520	6	336	4	308	4	407	5
418	6	247½	4	216	9	212½	7
420	6	253	4	261	7		
252	6	212½	6	420	7		
260	2	392	5	198	5		
125	2	135	4	160	6		
233½	3	240	5	252	6		
713	4	210	4	243	6		
		240	5				
		180	6				
		162	4				
		180	4				
		156	1				
		330	5				
		308	2				
Av...385	4.7	245.9	$4\frac{3}{18}$	238.7	$5\frac{1}{11}$	294	$5\frac{1}{5}$

[1] Average number of persons living or working on the premises.

We examined 22 nets (10 white, 10 colored, 2 flannel), taken from 10 different laundries, and found the following interesting contents:

TABLE 2.

[Contents of white nets; total number of nets, 10.]

Collars in 4 nets.
Shirts in 8 nets.
Nightshirts in 2 nets.
Undershirts in 7 nets.
Underdrawers in 10 nets.
Combination suits in 2 nets.
Pajamas in 2 nets.
Handkerchiefs in 9 nets.
Hose of all kinds in 5 nets.
Pillowcases in 3 nets.
Tablecloths in 3 nets.
Napkins in 4 nets.
Towels in 8 nets.
Bath towels in 2 nets.
Wash cloths in 3 nets.
Waists in 3 nets.
Aprons in 4 nets.
Chemises in 6 nets.
Corset covers in 3 nets.
Drawers in 4 nets.
Nightdresses in 8 nets.
Skirts in 6 nets.
Lace curtains in 2 nets.

[Contents of colored nets; total number of nets, 10.]

Shirts in 10 nets.
Nightshirts in 2 nets.
Undershirts in 9 nets.
Underdrawers in 9 nets.
Pajamas in 3 nets.
Handkerchiefs in 2 nets.
Hose in 3 nets.
Neckties in 1 net.
Towels in 3 nets.
Bath mats in 1 net.
Aprons in 3 nets.
Skirts in 1 net.
Waists in 4 nets.
Lace curtains in 1 net.
Bath robes in 1 net.
Dresses in 3 nets.
Wrappers in 1 net.
Overalls in 1 net.

[Contents of flannel nets; total number of nets, 2.]

White shirts in 2 nets.
Undershirts in 2 nets.
Underdrawers in 2 nets.
Combinations in 2 nets.
Hose in 2 nets.
Neckties in 1 net.
Aprons in 2 nets.

The contents of a few of the "nets" were listed and showed as follows:

TABLE 3.

RESUME OF CONTENTS OF NETS.

List of Articles.	White Nets.					Colored Nets.		Flannels.	
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.
Collars	1	..	..	..	..	..	..	..	..
Shirts	2	4	3	1	9	2	11	13	2
Nightshirts	..	..	3	..	1	..	..	..	..
Undershirts	8	4	6	..	8	1	5	5	11
Underdrawers	8	4	6	2	7	8	6	5	12
Combinations	..	..	..	..	..	..	..	1	2
Pajamas	..	..	..	1	..	..	1	..	..
Handkerchiefs	13	2	17	..	19	..	..	..	..
Hose	14	..	..	6	16	..	6	16	2
Neckties	..	..	..	..	..	..	..	1	..
Pillowcases	2	4	..	..	..	1	..	..	..
Tablecloths	3	..	1	..	..	..	..	..	..
Napkins	..	..	..	7	4	..	..	..	..
Towels	1	11	30	..	15	1	1	..	..
Bath Towels	..	..	15	11	..	..	..	..	..
Piece Muslin	..	..	1	..	..	..	..	..	..
Wash Cloth	..	..	1	7	..	..	..	..	..
Waists	1	..	..	..	2	7	1	..	..
Bath Mat	..	..	..	..	..	1	..	..	..
Aprons	..	2	..	..	..	..	..	2	3
Chemises	4	..	..	1	1	..	..	..	..
Corset Covers	..	..	2	..	2	..	..	..	..
Drawers	..	3	4	..	..	..	..	..	..
Nightdresses	2	1	4	3	1	..	..	..	..
Skirts	..	2	6	1	4	..	..	..	..
Lace Curtains	..	2	..	..	1	..	..	..	..
Doll's Cap	..	..	1	..	..	..	..	..	..
Guimpes	..	..	2	..	..	..	..	..	..
Bath Robe	..	..	..	..	..	1	..	..	..
Dresses	3	..	7	1	..	4	..	..	..
Wrappers	..	..	..	..	..	4	..	..	..
Piece of Lace	..	..	1	..	..	..	..	..	..

Washing—Of the 69 laundries forming the basis of this analysis, we found that 32, or 46.37 per cent., had absolutely no washing facilities on the premises. Of the 37 laundries which possessed such facilities, only 15 establishments washed clothes on the premises and that only to a limited extent. The entire group of 69 laundries were registered as sending their patrons' clothes to the steam laundries to be washed.

Drying Clothes—Upon completion of the washing process, most of the clothes are returned to the hand laundries wet.

The facilities for drying clothes indoors are most primitive—namely, lines strung below the ceilings or on racks run up on the side walls. The clothes are hung so closely on these lines that there is not sufficient opportunity for air, light, or heat to reach them effectively. These drying lines are placed in the store portion and also in the living quarters and when all the lines are in use the entire place is so filled that it is difficult to pass through the rooms. Frequently a stooping attitude must be assumed to avoid contact with the wet garments hung overhead.

Ironing—All flat work, i. e., sheets, pillowcases, bedspreads, towels, tablecloths, etc.—as a rule, is "finished" in the steam laundries. In some instances shirts, collars and cuffs are also "finished." The only work done by the hand laundries on this class of goods is to sort out and return the clothes belonging to the different patrons. All other garments are ironed by hand in the hand laundry.

In some establishments we found the store serving as laundry crowded with ironing benches and just enough room left to afford passage for a single person.

Summary Covering Hand Laundries—It will be seen from the foregoing data that the term "hand laundries" as understood by the general public, is a misnomer and that practically the only work done by hand is the receiving, marking, some ironing, and distributing.

If we then consider the facts just presented concerning the oftentimes crowded living quarters connected with the laundries, that cooking, eating and sleeping are carried on in close proximity to clothing intended for intimate wear by the general public; the probability of infectious diseases occurring among the persons living in such quarters without proper means of isolation, thus possibly furthering their spread among the patrons of the laundry; the slack methods employed for sorting clothes, allowing the contact of clean and soiled linen; the packing of nets with a heterogeneous mass of clothes too varied in color and type to permit the steam laundries to apply the proper degree of heat or amount of disinfectants necessary for good work and too closely packed to permit proper penetration of the wash water; the lack of proper facilities for sorting and drying the clean wet clothes received from the steam laundries; and, finally, the general insanitary conditions under which the work is carried on, it would seem that the hand laundries of the type inspected are to some degree a menace to the public health.

CHINESE HAND LAUNDRIES.

There are about 1,200 Chinese laundries scattered throughout New York City. Forty-three of these were inspected. There was a better division of the premises in these laundries than in those maintained by white people, the front of the store being in all cases devoted to the reception of clothes, marking, sorting and ironing, none of this work being done in the living quarters.

Division of Space—Eleven of the laundries had full partitions reaching to the ceiling and dividing the space, 8 had dwarf partitions and 15 laundries had both full and dwarf partitions. The use of the dwarf partitions provided for better ventilation.

Ventilation—The ventilation was good in 9 instances, fair in 8 instances and poor in 25.

Lighting—In 19 laundries there were good lighting facilities; 15 laundries had fair light; 8 laundries had poor light.

Condition of Premises—Eight of the laundries visited were clean, 12 were fair, 11 were poor. In the remaining 11 laundries no information was obtained on this point.

We found sleeping accommodations in 33, or 78 per cent. of the cases and have reason to believe that they may be found in all Chinese laundries. The highest number of bunks in any one laundry was found to be four. The rear of the store was usually divided into two portions, one portion being devoted to the washing of the clothes and cooking. The other portion was, as a rule, inclosed by full partitions and served as a drying room.

All of the laundrymen admitted the cooking of food and its consumption on the premises.

Methods Employed in Handling Clothes—Marking and Sorting—Soiled linen was usually received over a counter specially devoted to it and marked by hand. In no instance was soiled linen found in contact with clean linen. The sorting of clean linen is done on the ironing tables which are always clean.

Washing—All washing is done by hand on the premises, the clothes being laid upon a board and scrubbed with stiff brushes. A few cradle washing machines were found. In every laundry visited large copper boilers were in evidence which, according to statements made to us, were used in boiling the white clothes. A few temperatures were taken of these boilers while in operation and it was found that the temperature of the water varied from 180 to 210° F. The colored clothes and flannels are not boiled being subjected merely to warm or hot water rinses.

Drying—All the laundries visited had a drying room. The center of the room was occupied by a stove usually red hot. Unfortunately the temperature of these rooms was not taken. The clothes were strung below the ceiling and along the side walls.

Dampening the Clothes—Three types of dampeners were found in use:

Type 1 was the ordinary large rubber bulb used by the florists for flowers.

Type 2 was a can supplied with two openings, spray and mouthpiece. The water in the can was forced out in the form of a spray by blowing into the mouthpiece.

Type 3 was simply a whisk broom used to sprinkle the clothes by whisking water over them.

Types 1 and 3 fulfilled their purpose admirably without infecting the clothes in any way, but were infrequently used. Type 2 was in evidence in every establishment visited and has in it the elements of danger when handled by a person having an infectious disease like tuberculosis, syphilis (mucous patches), diphtheria, etc., as infection might possibly take place. The can is used indiscriminately by the employees.

Upon completion of the ironing processes, the clean clothes are hung on lines to dry. When dry, they are gathered together on the large clean ironing tables where they are sorted and wrapped. In no instance did we find clean and soiled linen in contact.

PART II.—STEAM LAUNDRIES.

The occasional report concerning the suspected transmission of disease through the medium of the laundries and the lack of sufficient sanitary regulations concerning the methods employed in steam laundries led to this brief study of some of the problems involved.

The investigation covered practically all of the prominent steam laundries in New York City.

The bacteriological methods employed were:

1. The testing of the bactericidal action of water plus the soap and disinfecting solutions and the possible mechanical elimination of large numbers of bacteria in the process of washing.
2. The testing of the penetrative power of the heat employed in washing.
3. The testing of the value of the ironing processes in the destruction of bacteria.
4. The testing of the bactericidal value of the drying processes.

The testing of the bactericidal and eliminative action of the water used in the cleansing processes was effected in three ways:

(a) By the addition of enormous numbers of *Bacillus coli* to the wash water and the taking of samples of the water for bacterial analysis at stated intervals throughout the process of washing.

(b) By the routine bacteriological examination of a large number of wash waters taken throughout the various steps of the washing process.

(c) By the use of strips of cloth of various types which had been previously sterilized and soaked in horse serum to insure a viscous covering and then inoculated with pure cultures of *Bacillus coli;* These prepared strips were placed in small laundry bags and washed with the clothes.

Testing of the Penetrative Power of the Degree of Heat Employed.

To test this factor, small containers were devised. These consisted of pieces of rubber tubing one-fourth inch in diameter and about 5 inches in length. The ends were fitted with "no-air" rubber stoppers. When required for a test, the tubes closed at one end were sterilized by boiling for half an hour and then filled with a 24-hour culture of *Bacillus coli* in broth. The open end was then stoppered and both ends were capped with sterile capping skin. The tubes thus prepared were inclosed in towels, tied and placed in the center of the washing wheel among the garments being washed. Maximum thermometers tied in a similar fashion were also used.

Testing the Bactericidal Value of the Ironing Processes.

This was accomplished by infecting various types of garments with cultures of colon bacilli and subjecting them to the action of the different forms of irons and presses used. (See ironing.)

Testing the Bactericidal Value of the Dry Houses and Drying Tumblers.

This was done in two ways—by hanging strips of infected material in the dry houses and then testing these strips bacteriologically; also by the use of maximum thermometers which were so placed among the garments that they passed through the dry house simultaneously with the clothes and thus registered the maximum degree of heat to which the clothes were subjected.

All of these tests were made at the various types of steam laundries and then the testing material was transported to the laboratory where the bacteriological analysis was made, the samples being kept iced during transit. The culture media employed were plain agar for the quantitative count and Endo's medium and lactose neutral red fermentation tubes for the qualitative presumptive test for *B. coli.*

The incubation period of the plates was 48 hours at 37° C.; of the fermentation tubes, three days at 37° C.

A special card for the collection of data concerning the washing processes was also prepared.

Sanitary Survey and Bacteriological Studies.

There are approximately 125 steam laundries scattered throughout New York City exclusive of the laundries attached to institutions and hotels. They may be roughly classified into four groups:

Group 1. Laundries connected with infectious disease hospitals.

Group 2. Laundries which deal directly with families or individual patrons in which the clothes are washed and finished.

Group 2. Laundries which deal directly with families or individual patrons through the medium of hand laundries.

Group 4. Wet wash laundries.

The methods employed in the infectious disease hospitals' laundries are distinctly different from those empolyed in an ordinary public laundry, and will, therefore, be described separately. Groups 2, 3, and 4 will be considered collectively.

Group 1. Management of Linen for Contagious-Disease Hospitals.

New York City possesses at present two laundry centers dealing with linen which is known to be infected to a considerable extent. These laundry centers are situated at the Kingston Avenue Hospital in Brooklyn and at the Riverside Hospital on North Brothers Island.

These laundries take care of all the soiled clothes from 59 milk stations; 15 eye and dental school clinics; 3 ambulance stations; 3 venereal disease clinics; 3 hospital plants containing 2,310 beds; and the Bureau of Laboratories. They handle approximately 11,900 pieces per day.

Owing to the infectious nature of the material handled, the laundries are so arranged that a partition separates the sorting room from the rest of the building and permits the loading of the washing wheels through chutes leading from the sorting room directly into the washers.

Sorting—The sorting is done by workers who are attired in a special gown having long, loose, closed sleeves which cover the hands. The hair is covered with a cap. This uniform was adopted two years ago. Since then there has been no case of infection among the sorters.

Washing—The washing is done in the regular compartment washers, which are fitted with thermometers registering the temperature of the water.

A summary has been prepared giving the types of clothes washed, the number of processes employed in washing, the length of time in minutes in which clothes were exposed to the action of steam, the total amount of time used in washing and the manner in which the clothes were finished:

TABLE 4.

SUMMARY OF METHODS OF LAUNDERING CLOTHES (CONTAGIOUS DISEASE HOSPITALS).

Type of Goods.	Number of Processes Employed in Washing.	Time Exposed to Steam.	Total Time Used in Washing.	How Finished.
		Minutes.	Minutes.	
Bedclothes, Bath Towels	5	30	50	Mangle.
Clinic Gowns	5	30	50	Mangle.
Uniforms, Doctors', Nurses'	5	60	90	Ironed by hand.
Table Linen, Napkins, Waitresses' Aprons	5	45	65–70	Ironed by hand and mangle.
Colored Goods—Maids' Aprons, Uniforms	4	45	60	Ironed by hand.
Overalls, Jumpers, Socks, Visitors' Gowns, Covers, Shirts	4	10[1]	25	Roughly sent through dry house, 250° F.
Flannel Blankets	4	15–20[1]	25–30	In dry house.

[1] Hot water only; no steam.

All clothes were subjected to the action of high temperatures during the process of either washing or drying. A few of the temperatures used in the washing machines are shown in Table 5:

TABLE 5.

TABLE SHOWING TEMPERATURES.

Number of the Washing Wheel.	Nature of Goods Washed.	Method of Washing.	Process of Washing when Temperatures were Taken.	Temperaature.	Method of Finishing
				°F.	
1	Bed Linen	Loose	Beginning of Hot Suds	165	Mangle.
2	Bed Linen	Loose	End of Hot Suds	. . .	Mangle.
3	Bed Linen	Loose	First Hot Suds	100	Mangle.
4	Diapers	Loose	Beginning of Hot Suds	180	Mangle.
5	Diapers	Loose	End of Hot Suds	200	Mangle.
6	Pajamas	Loose	Beginning of Hot Suds	175	Dry house (214 °F).
7	Underwear	Loose	End of Hot Suds	190	Dry house (214 °F).

A review of the methods employed leads one conclusively to the belief that the destruction of pathogenic organisms was insured.

Analysis of Groups 2, 3 *and* 4

As to the location and housing of laundries, the conditions varied greatly from the first-class laundry, properly housed and equipped in a most sanitary, efficient manner, to the wet-wash laundry, located in a subcellar and conducted under the most insanitary conditions. This irregularity of type also existed throughout in the methods employed for handling the clothes.

Collection of Clothes—As a rule, collections and deliveries are made by the same wagon, only the first-class laundries maintaining separate services. Some of the laundry wagons carry clean and soiled linen promiscuously, delivering clean linen and collecting soiled.

Receiving and Sorting Clothes—In the average laundry the clothes packed in bundles or large nets were received either in the wash room or in the sorting room, where clean and soiled linen were in close proximity and handled in a more or less insanitary manner. Insanitary surroundings seemed particularly prevalent in the wet-wash laundries visited. In the better-class laundries, in which the clothes were finished and which dealt directly with families and individual patrons, we found receiving rooms partitioned off from the wash room either by railings or dwarf partitions. The sorting and marking of the linen is done on tables fitted either with stalls and box compartments over the table level or bags attached to the side. Some laundries sort directly into bins or trucks.

Clothes obviously infected with vermin, or known to have come from a house where an infectious disease is said to be present, or clothes excessively polluted are either refused and returned to the person sending them or else washed and handled separately in what is known as the "dirty-clothes washer." The watch for vermin-infected clothes is kept rather sharply, as these pests have been known to infect an entire laundry and cause severe financial loss.

Washing Methods.

Machinery Employed in Washing Clothes—The routine washing is done in regulation washing wheels or "washers" which vary in size from one measuring 32 by 36 inches to one measuring 42 by 96 inches.

The "washer" consists of a double cylinder made of wood, copper, or brass so arranged as to furnish a double rotary motion which subjects the clothes to an alternate squeeze and rinse or splash movement. The inner shell is perforated and permits the free access of water to the clothes during the washing process. The interior of this shell is divided into one or more compartments by vertical and transverse partitions by means of which different lots of clothes are held apart though subjected to the action of the same wash water. These partitions also serve to diminish friction. From 2 to 10 lots may be contained in the same washer.

Some of the washers are built so that the water enters into them either cold or lukewarm and is heated by means of steam to any desired temperature. This method is used in the disinfecting sterilizers and in many of the earlier machines.

A new system coming rapidly into use on account of its convenience and economy is that in which the water is heated in large combination tanks by means of brass coils containing superheated steam. The heated water is then piped directly to the washing machines as required. This latter method eliminates the separate steam connections necessary in earlier types of washing machines.

Methods Employed in Washing—As a rule the processes employed vary according to the types of goods being washed, the amount of clothes in the washer and the amount of soil upon the garments, which may require an increase or lessening of the amounts of washing material used. The personal standards of the foreman of the washmen are important factors also as he is absolute in his domain.

In loose washing the clothes are handled practically as clothes at home are managed. This method is in use in first-class laundries which deal directly with families or individual patrons. We found that as a rule the white goods which were washed "loose" received a sufficient amount of disinfectants and heat to practicallly destroy pathogenic bacteria.

A brief summary of the methods employed in washing white goods "loose," together with the results obtained in testing the processes bacteriologically, is given in Table 6. This table is interesting, as it shows the effect of various processes, temperature, and time upon pathogenic bacteria in clothes. We have reason, however, to believe that this table does not represent the actual conditions usually employed by the laundrymen, but that their zeal to obtain a perfect score led them into washing overtime.

The effect of the heat exposures was tested by the use of the containers described on page 433. We found that even with the lowest temperature and shortest exposure tabulated the colon bacilli (which approximated 500,000,000 per c. c.) were invariably killed.

TABLE 6.

WHITE GOODS WASHED LOOSE.

Type of Goods Washed.	Number of Processes Employed; Breakers, Suds, Rinses.	Total Amount of Time Consumed.		Maximum Temperature Obtained.	Length of Exposure to maximum Temperature.	Bacteriological Analysis.				
						Water Samples at End of First Rinse.		Water Samples at End of Last Hot Suds.		Broth Culture in Rubber Tubes Inserted Containing 500,000,000 B. Coli per c.c. Contents Tested at End of Process.
						Bacteria per c.c. Nutrient Agar.	Bacteria B. Coli Group in c.c. l. n. r. f. t.[1]—	Bacteria per c.c. Agar.	Bacteria B. Coli Group in c.c. l. n. r. f. t.[1]—	
		Hrs.	Min.	° F.	Min.				c.c.	
Family Wash...	11		45	181.9	20	6,730,000	+ in 1/10000	57	—1	Sterile
Family Wash...	7	1	32	170.0	45	1,600,000	+ in 1/7000	150	—1	Sterile
Family Wash...	6	1	35	167.0	30	9,000,000	+ in 1/1000	150	—1	Sterile
Family Wash...	5	1	25	170.0	35	28,000,000	+ in 1/1000	350	—1	Sterile
Flat Goods....	6	1	6	140.0	26	16,200,000	+ in 1/100	70	—1	Sterile
Flat Goods....	6	1	10	158.0	40	374,000	+ in 1/10000	80	—1	Sterile
Flat Goods....	7	1	33	156.0	38	13,000,000	+ in 1/100	150	—1	Sterile
White Goods...	6	1	35	167.0	40	18,000,000	+ in 1/1000	30	—1	Sterile
White Goods...	5		55	170.0	25	90,000	+ in 1/1000	50	—1	Sterile
White Aprons..	7	1	35	124.0	30	1,560,000	+ in 1/1000	100	—1	Sterile
Aprons........	7	1	50	124.0	8	1,500,000	+ in 1/1000	750	—1	Sterile
Collars........	10	1	50	151.0	16	300,000	+ in 1/1000	7,000	—1	Sterile
Collars........	10	2	27	185.0	30	1,700,000	+ in 1/1000	...	...	Sterile
White Shirts...	9	1	50	158.0	30	14,000,000	+ in 1/1000	600	...	Sterile
White Shirts...	8	1	10	149.0	23	259,000	+ in 1/10	...	...	Sterile
Towels........	9	1	50	138.6	45	483,000	+ in 1/10000	200	...	Sterile

[1] Lactose neutral red formation tubes.

The material washed according to the "loose" method forms a comparatively small percentage of the clothes received by steam laundries. Fully 90 per cent of the clothes washed in the steam laundries of New York City are washed in "nets," and this presents a most serious problem.

Net Washing—"Nets" vary in size from 18 by 20 inches to 42 by 48 inches. When packed they weigh from 10 to 35 pounds dry and 60 to 80 pounds wet. At present they are being used by the hand laundries in an unsuitable manner.

Thirty expert laundrymen and owners frankly stated that in their opinion the system of washing clothes in large, tightly packed nets is responsible for the following evils:

First. That the soapsuds employed are immediately "cut"[1] and lose their efficiency.

Second. That the water does not penetrate properly to the center of the nets in the time allotted to the washing process.

Third. That owing to the miscellaneous character of the contents of the nets it is impossible to treat them with a sufficient amount of disinfectants or heat to destroy the pathogenic bacteria and vermin.

In a series of tests made by us in laundries it was found that sugar and salt wrapped up in packages and placed in the center of tightly packed nets were only partially melted, and we have in some instances recovered over 50 per cent of this material unmelted after the packed nets had been washed the regulation period.

Washing Colored Clothes—Colored clothes are washed in the same type of machines as the white clothes. One method is the cold process where cold or lukewarm water is employed. The second or hot method approaches closely the method employed in washing white clothes.

The main points of difference are these: First, lower temperatures are used to prevent the fading of the clothes; second, fewer processes are employed.

Of the 80 laundries investigated where positive information could be obtained upon this latter point it was found that the number of processes used for colored clothes varied from two to eight. If only two processes were used, they consisted of a lukewarm suds followed by a cold rinse. If the clothes were treated with three or more processes, the additions usually consisted of a cold rinse and second hot suds followed in the better-class laundries by an increased number of rinses. For example, in a place using five processes the method of procedure was as follows: 1, cold rinse; 2, hot suds; 3, hot rinse; 4, cold rinse; 5, cold rinse with blue.

The statistics gathered are grouped together in Table 7.

TABLE 7.

SUMMARY OF METHODS EMPLOYED IN WASHING COLORED GOODS.

COLORED GOODS WASHED LOOSE AND SUBJECTED TO INFLUENCE OF DRYING HOUSE OR TUMBLER

Number of Laundries.	Total Number Processes Employed.	Average Time Consumed, All Processes.	Details of Processes—Average Time.			Washing Classified According to Type—Method Used.			
			Breaker Process.	Suds.	Rinses.	Cold.	Lukewarm.	Warm.	Hot.
		Minutes.	Minutes.	Minutes.	Minutes.				
7.	4	54	6	26	22	1	. . .	5	1
8.	5	71	12.5	33	17.5	. . .	1	2	5
2.	6	70	7.5	17.5	40	. . .	. . .	1	1
4.	7	66.5	10	37.5	26.5	. . .	. . .	. . .	4
Total 21 (Average). . . .	5.5	65.3	9	28.5	26.5	. . .	. . .	. . .	. . .

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[1] Term used by laundrymen to note the condition which results when an excessive amount of soil is brought into contact with soapsuds.

TABLE 7—Continued

Colored Goods Washed Loose and Returned to Patrons Wet.

3.	3	33	1.5	21.6	10	. . .	2	1	. . .
3.	4	55	5	16.6	16.6	. . .	1	2	. . .
11.	5	58	15	24	17.8	. . .	3	5	3
2.	6	87	12.5	45	32	. . .	. . .	1	1
Total 19 (Average). . . .	4.5	58.21	8.2	26.8	19.4	. . .	. . .	. . .	. . .

Colored Goods Washed in Nets and Returned to Hand Laundries Wet.

2.	4	42.5	7.5	17.5	12.5	. . .	. . .	2	. . .
7.	5	60.5	13.7	25.7	20.7	. . .	. . .	5	1
2.	6	62.5	7.5	32.5	22.5	. . .	. . .	1	1
1.	7	80	10	50	20	. . .	. . .	1	1
2.	8	82.5	10	62.5	40	. . .	. . .	2	. . .
Total 14 (Average). . . .	6	65.6	9.7	37.6	23.1	. . .	. . .	. . .	. . .

These figures show that 40 of the steam laundries visited washed the colored goods according to the "loose" method. Of these 40 laundries, 21 laundries dried the clothes in the drying house or tumbler where they were subjected to a temperature of 210° F. or over, and 19 of the laundries returned the goods to the patrons wet. Fourteen steam laundries washed the colored goods in nets and returned them to the patrons wet. There were thus 33, or 58.9 per cent. of the laundries visited which returned the colored clothes to the patrons wet. It may therefore be stated that practically only 38.8 per cent. of the colored goods were "safe" when considered from a bacteriological point of view.

The average number of processes employed in washing and the final disposal of the garments varied as follows:

TABLE 8.

Number of Laundries.	Average Number of Processes.	Final Disposal of Goods.
21.	5.5	Sent to dry house.
19.	4.5	Sent out wet.
14.	6.0	Sent out wet.

The average time consumed in washing was 65.3 minutes for clothes washed loose and sent to the "dry house" or "tumbler"; 58.2 minutes for clothes washed loose and sent out wet, and 65.6 minutes for colored clothes washed in nets and sent to hand laundries wet.

Tests of Washing Machines.

As a great deal of stress is commonly laid upon the benefits derived in washing from the rotary motion of the washers, a series of tests was made to ascertain just how much movement took place when the "washers" were tightly packed with heavy nets. The testing nets were therefore marked and placed in the center and at each end of the "washer." It was ascertained that there was a very slight amount of lateral displacement in the end nets and no change of position in the center nets.

Study of Soaps, Bleaches, and Disinfectants Employed.

Using the methods employed by the United States Public Health Service, approximately 50 tests were made of the soaps, bleaches, and disinfectants used in washing. A study of the soap solutions used showed that they possessed no germicidal value in the strengths employed. In one instance we were able to isolate a staphylococcus from one of the strong soap solutions which is ordinarily 10 times as strong as the solution used in the washing machine.

In the study of the bleaches, we found that the average bleach and disinfectant used required an average strength of at least 1 per cent. to prove effective in destroying *Bacillus coli* in 30 minutes. This percentage strength can not be used in the actual work for two reasons—one is the prohibitive cost, the other is the corrosive action upon the clothes. One of the large heavy and new canvas bags used in making certain washing tests, on its third trip through the "washer" showed the effect of the caustic solution improperly applied to the clothes when a large hole suddenly appeared in the material.

The Handling of Woolens.

To ascertain the methods used in washing woolen goods, information was obtained from 31 laundries. The data obtained in these places applies only to the better grades of woolen underwear, as fully 90 per cent. of the ordinary underwear is washed in nets. In 13 of these laundries the flannels were washed individually by hand and the time devoted to each garment varied according to its size and condition, the approximate time varying from 5 to 10 minutes per garment. An analysis of the figures obtained showed that 2 laundries used 2 processes; 8 laundries used 3 processes; 2 laundries used 4 processes; 1 laundry used 5 processes.

Eight of the laundries, or 61.5 per cent., dried the flannels in a "dry house." In a number of instances, however, where flannels were concerned, the drying house was found to have a comparatively low temperature, 120° F., and bacteriological tests made during our investigation did not show any perceptible destruction of bacteria.

A review of the data obtained from 18 laundries where no hand washing was done is summarized as follows:

One laundry used 2 processes with an average of 20 minutes' washing time.

Five laundries used 3 processes with an average of 39 minutes' washing time.

Two laundries used 4 processes with an average of 50 minutes' washing time.

Six laundries used 5 processes with an average of 52 minutes' washing time.

Three laundries used 6 processes with an average of 37.5 minutes' washing time.

One laundry used 7 processes with an average of 80 minutes' washing time.

In this group 5, or 27.7 per cent., returned the flannels to the patrons wet; 4, or 22.2 per cent., dried them in the open air; and 9, or only 50 per cent., subjected them to temperatures high enough to destroy ordinary pathogenic bacteria.

If we remember the possibilities of the presence of infectious material upon flannels and that they are practically never subjected to the same temperatures and methods employed for the average white wash, it can be readily seen that they offer a serious sanitary problem.

When the washing processes have been completed, the clothes, whether washed loose or in "nets," are placed in the "extractors." These machines are so constructed that the inner basket revolves at a high rate of speed and the removal of the excess water in the garments takes place through centrifugal force.

Comparatively few of the garments cleansed in steam laundries are dried and ironed upon the premises. This is due to the fact that practically 90 per cent of the clothes are washed in nets. These nets are taken from the extractors and returned to the hand laundries wet and unopened.

In the so-called "wet-wash" laundries which devote themselves exclusively to "family wash," the cleansing processes also terminate with the use of the extractors. In a number of the places visited the clothes were placed in a clean basket or bag and sent back to the patrons, thus giving a false sense of security through a belief in the efficiency of the laundry methods.

The foregoing data concerning the methods employed in washing of the nets, colored goods, and flannels, were submitted to a group of laundrymen and they practically agreed that the time and temperature elements in the cases observed were somewhat higher than those usually employed by steam laundries. They stated that the average time limit was from 35 to 45 minutes and the average temperature varied from 90 to 110° F.

Experimental work—To test the efficiency of the washing processes when restricted as to time and temperature, but not limited in regard to soaps, bleaches, or water, a series of laboratory experiments was arranged. The testing materials consisted of strips of half woolen and woolen goods 6 by 9 inches. These strips were dipped into water containing *Bacillus coli* and approximating 8,120,000 bacteria per cubic centimeter. The prepared tests used in the earlier experiments were employed also and filled with *coli* broth which contained approximately 812,000,000 per cubic centimeter. The inoculated material and the tubes were wrapped in towels, tied and placed among the clothes which were to be passed through the washing processes.

The machine was filled with small nets weighing approximately 2 pounds each. The washing was then done as follows: 2 cold rinses, 5 minutes each; 1 hot suds, 10 minutes duration; total amount of time, 20 minutes.

The result of these experiments showed comparatively slight reduction of the bacteria in either the prepared tests or in the broth tubes of the watery suspension.

A second series of experiments was worked out with an increased time limit (54 minutes for total processes and a temperature limit of 93.5° F.). This, as we had been assured by the laundrymen, represented the average temperature plus a slightly increased time limit employed for colored clothes and flannels. No bactericidal destruction took place.

A third series of experiments with the same time limit but at higher temperature was also tried. With packed nets averaging 25 pounds each and a temperature of 150° F., there was a reduction of 50 per cent in the number of bacteria present in the tests inclosed in the center of the nets.

These experiments show conclusively that the temperature and time limits employed were wholly inadequate to destroy bacteria of the pathogenic type.

Methods Employed in Finishing Goods.

Clothes finished in steam laundries are handled in one of three ways—roughly dried, mangled, ironed by hand or machine.

Rough dry or family wash—In order to eliminate the evils of the nets and wet-wash systems, a number of the better-class laundries have begun to establish what is known as "rough-dry," "pound," or "family wash."

The articles in this type of washing are taken from the "extractors" and sent through the drying houses or tumblers where they are subjected to a high temperature, 210 to 250° F. A series of tests was made of a number of drying houses and tumblers to ascertain the exact temperatures and the results are given in Table 9.

TABLE 9.

TIME AND TEMPERATURE EMPLOYED IN LAUNDRIES IN THE DRYING HOUSES.

Type of Drier.	Type of Clothes Dried.	Time.	Maximum Temperature.
		Minutes.	°F.
Metal Lined, Steam Coils	Colored Goods	10	210
	Colored Goods	30	270
Metal Lined, Chain Conveyors, Steam Coils	Colored Shirts	30	275
	Collars	15	210
	Flannels	30	275
Metal Lined, Sliding Racks, Steam Coils	Flannels	30	210
	Colored Shirts	50	212
Metal Lined, Metal Sliding Racks, Steam Coils	Colored Shirts	30	225
	Colored Shirts	20	225
Automatic Chain Drier, 34 Minutes	Collars	45	200
	Colored Shirts	55	180
	Flannels	55	180
Metal Lined, Iron Sliding Racks, Steam Coils	Bath Towels	20	225
	Bath Towels	30	225
Automatic Chain Drier, Metal Racks	Collars	20	270
	Flannels	30	268
Metal Lined, Steam Coils	Flannels	35	251
	Colored Shirts	32	285
Metal Lined, Metal Racks, Steam Coils	Blankets	40	212
	Colored Goods	30	210
Galvanized Iron, Red-hot Coal Stove, Copper Wires Across Room	General Wash	25	200
	General Wash	20	200

The lowest temperature maintained in the dry rooms was 180° F.; the highest, 285° F.; and the average, 226.7° F. The shortest period of time employed in drying was 10 minutes at 210° F.; the longest period of time employed in drying was 55 minutes at 180° F. The average time employed was 29.5 minutes, 226.7° F.

Of the 22 dry rooms tested, 20 maintained a temperature of 200° F. or over. This degree of heat, together with the steam arising from the wet garments, insures the destruction of all bacterial and animal life. The additional cost which this drying entails is comparatively slight, ranging from one-half to 2 cents per pound.

Methods Employed in Ironing Clothes.

Goods finished through use of mangle.—Mangles are simply large iron rollers so arranged as to offer a large heating and pressing surface. These presses are employed to iron knit underwear, sheets, pillowcases, bedspreads, towels, tablecloths, napkins, etc.

Strips of various types of material (cotton, cotton and wool, wool) were immersed in coli broth and sent through the mangles. As a result of these tests we found that if the mangles were properly heated and run at the right speed, one passage through the rollers was sufficient to destroy bacterial life upon cotton goods. The tests of the wools showed that it was necessary in some instances to send the tests through at least twice to insure the complete bactericidal effect of the heat. A few of the experiments are given in Table 10.

Ironing, hand—A number of tests were made to ascertain the temperature of the hand irons. The result of the tests showed that the degree of heat requisite to insure the proper smoothness was sufficient to destroy bacterial life. The danger in hand ironing of clothes which have not been subjected to high temperatures during the washing or drying processes lies in the fact that double edges and seams which may contain infectious material, are apt to be overlooked.

Machine ironing—To ascertain the efficiency of machine ironing, articles of clothes soaked with *Bacillus coli* were ironed in the usual manner and then examined. The bacteriological analysis showed that the machines employed an effective degree of heat.

Since the collection of the foregoing data New York City has suffered from an epidemic of poliomyelitis (infantile paralysis) and it was thought worth while to ascertain if contagion had been conveyed through laundries. Fifty-five cases taken at random were investigated. It was found that the majority of the families did not send their laundry out of the homes. In a few instances it was ascertained that a number of the afflicted families had patronized the wet-wash laundries, but no direct contagion could be traced to them. The better class of steam laundries refused to handle the clothes which were known to have come from infected houses.

TABLE 10.

MANGLE TEST.

Number of Test.	Type of Material Used in Test.	Number of Times Through Mangle.	Time Passing Through Rollers.		Bacteriological Results.		
					Endo Plates, Bacteria per c.c.		Members of B. Coli Group in—
					Before Test (Approximately).	After Test.	
			M.	S.	Per c.c.		c.c.
1	Half Wool	Once		80	500,000,000	Sterile	— $\frac{1}{10}$
2	Half Wool	Once		40	500,000,000	Sterile	—1
3	Half Wool	Once		45	500,000,000	Sterile	—1
4	Half Wool	Twice		70	500,000,000	Sterile	—1
5	Half Wool	Twice	2	40	500,000,000	Sterile	—1
6	Half Wool	3 Times	4	50	500,000,000	Sterile	—1
7	Half Wool	3 Times	2	37	500,000,000	Sterile	—1
8	Half Wool	3 Times	1	36	500,000,000	Sterile	—1
9	All Wool	Once	1	20	500,000,000	Sterile	—1
10	All Wool	Once		40	500,000,000	Sterile	—1
11	All Wool	Once		45	500,000,000	Sterile	—1
12	All Wool	Once		70	500,000,000	Sterile	—1
13	All Wool	Twice	2	40	500,000,000	Sterile	—1
14	All Wool	3 Times	4	50	500,000,000	Sterile	—1
15	All Wool	3 Times	2	37	500,000,000	Sterile	—1
16	All Wool	3 Times	1	36	500,000,000	Sterile	—1
17	Half Wool[1]	Once		45	500,000,000	Sterile	—1
18	Half Wool[1]	Once	2	15	500,000,000	Sterile	—1
19	Half Wool[1]	Twice	1	55	500,000,000	Sterile	—1
20	Half Wool[1]	Twice	1	10	500,000,000	Sterile	—1
21	Half Wool[1]	Twice	2	40	500,000,000	Sterile	—0
22	Half Wool[1]	3 Times	4	50	500,000,000	Sterile	—1
23	Half Wool[1]	3 Times	2	37	500,000,000	Sterile	—1
24	Half Wool[1]	3 Times	1	36	500,000,000	Sterile	—1
25	All Wool[1]	Once		45	500,000,000	Sterile	—1
26	All Wool[1]	Once	2	15	500,000,000	Sterile	—1
27	All Wool	Twice	2	40	500,000,000	Sterile	—1
28	All Wool	Twice	1	10	500,000,000	Sterile	—1
29	All Wool	Twice	1	55	500,000,000	Sterile	—1
30	All Wool	3 Times	4	50	500,000,000	Sterile	—1
31	All Wool	3 Times	2	37	500,000,000	Sterile	—1
32	All Wool	3 Times	1	36	500,000,000	Sterile	—1
33	Half Wool	Once	1	15	500,000,000	245	+1
34	Half Wool	Twice	2	30	500,000,000	1,576	+1
35	Half Wool	3 Times	3	45	500,000,000	......	—1
36	Half Wool	4 Times	5		500,000,000	......	—1
37	All Wool	Once	1	15	500,000,000	1	—1
38	All Wool	Twice	2	30	500,000,000	200-3,240	+1
39	All Wool	3 Times	3	45	500,000,000	25,000	+1
40	All Wool	4 Times	5		500,000,000	......	+1

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[1] Strip dipped in cottonseed oil before inoculation.

SUMMARY.

Hand Laundries.

1. The sanitary conditions existing in the average "hand laundry" managed by white persons are of a very low grade, falling far below those existing in Chinese laundries.

2. The State factory laws concerning living quarters are violated in both Chinese and hand laundries.

3. All clothes sent to the average Chinese laundries are washed and dried on the premises, in separate rooms maintained for this purpose.

4. The use of the "blow can" for dampening clothes is universal in Chinese laundries and may lead to infection.

5. The methods of marking and sorting the clothes employed in the average "hand laundry" are unsuitable and a possible source of danger to the health of the employees and community.

6. Very little washing is done in the average hand laundry. The practice now prevails of tightly packing a heterogeneous collection of soiled clothes into large bags or nets. These nets are then sent to the steam laundries where they are washed as units and returned to the hand laundries wet.

7. The drying facilities employed in the hand laundries are limited and of a very primitive type. The process is not such as would secure the death of all the pathogenic germs which may have survived the washing process.

Steam Laundries.

8. The comparatively few steam laundries which use standard routine methods of washing and keep a record of the time, material and solutions employed are getting better results than the average establishments.

9. The methods employed by steam laundries in the collection and delivery of clothes are found to be defective in many respects in a considerable proportion of the laundries. Soiled and clean clothes are carried on the same wagon and come into direct contact with each other. They are also frequently sorted in close proximity as comparatively few establishments maintain separate receiving rooms where the clothes may be properly handled.

10. In the majority of laundries the clothes are washed under conditions prejudicial to the health of the employees, the "washers" being usually located in basements poorly lighted and ventilated with defective floors and without adequate provisions for the disposal of waste water and steam.

11. The method at present commonly employed by certain steam laundries of returning "wet" clothes to the patrons and to hand laundries is a possible menace to the public health.

12. Wet clothes infected with bacteria and subjected to the action of the usual degree of heat found in drying houses, tumblers, mangles and hot presses are freed from living organisms.

13. The practice of "net washing" as now done in steam laundries is insanitary. The miscellaneous character of the contents of the nets prevents the proper application of disinfectants, soap, water and heat and thus permits the survival of vermin and pathogenic organisms. The size of the nets and the methods of tight packing employed prevent the penetration of water and heat in the allotted time.

14. Owing to the difficulty of ascertaining whether clothes have been properly heated during the washing processes and the possibility of the transmission of infection when not properly treated, all clothes washed in steam laundries should be dried upon the premises.

15. The absence in the average steam laundry of proper sorting rooms for the clean linen and the consequent contact with soiled linen may result in a possible reinfection of the clean clothes.

These studies were taken up at the suggestion of the Public Health Committee of the Association for Improving the Condition of the Poor, and we had their cooperation in the early part of the work. We are also indebted to the Brooklyn Steam Laundrymen's Association for their aid in procuring valuable data.

INFANTILE SCURVY

III. Its Influence on Growth (Length and Weight).*

ALFRED F. HESS.

In two previous papers[1] on this subject it was shown that pasteurized milk, that is, milk heated to 145 F. for thirty minutes, gradually induces infantile scurvy, unless antiscorbutic diet is given in addition. The fact that this disorder quickly yielded to the substitution of raw for pasteurized milk, or to the addition to the diet of an ounce of orange juice, or the juice of orange peel, was regarded as satisfactory evidence of the true scorbutic nature of the disturbance. The type of malnutrition which gradually develops from a diet of pasteurized milk may be termed subacute scurvy, as it takes some months to develop and, as a rule, does not manifest the pronounced symptoms characteristic of the classic case. In some instances, however, we encounter subperiosteal hemorrhages and the hemorrhagic gums typical of this disorder. That infantile scurvy is not met with more commonly, in view of the widespread use of pasteurized milk as a food for infants, may be ascribed to the fact that orange juice or other antiscorbutic food is so generally given in addition to milk. In other words, although pasteurized milk is to be recommended on account of the safety which it affords, it must be regarded as an incomplete food for infants. That the disease developing under these conditions is subacute in nature is to be attributed to the fact that the "vitamins," which are not overabundant in commercial milk, are not entirely destroyed by pasteurization, so that the infant receives day by day a small amount of these essential substances.

It will be remembered that the cases of scurvy discussed in the previous articles arose in an institution where the use of orange juice was discontinued, in view of the report of the Commission on Milk Standards to the effect that milk does not suffer a destruction of enzymes or other chemical constituents in the course of pasteurization. In the first paper the nature of the hemorrhagic condition was studied as to the question of involvement of the blood or of the blood vessels. In the subsequent paper the symptoms of infantile scurvy were shown to bear an intimate relationship to those of other deficiency diseases, more particularly beriberi. It was pointed out that scurvy should no longer be regarded clinically as a disorder characterized merely by hemorrhages, nor from a pathologic viewpoint merely as one manifesting changes in the bones, but that signs and symptoms of involvement of the heart (enlargement of the right ventricle and tachycardia) and of the peripheral nerves are also evident, so that a broader aspect is demanded. This paper, the third in the series, which is based mainly on the same cases which formed the groundwork of the previous studies, shows how these infants reacted in their growth to alterations in diet; how they grew on a diet consisting of pasteurized milk, sugar and cereal, when they were at the same time receiving orange juice; when the orange juice was discontinued, and when this juice or the juice of the orange peel was again added to the diet. These infants offered a particularly favorable opportunity for observations of this kind because they entered the institution at an early age and remained there for one or more years, and also because they were most carefully observed, weighed daily and measured on admission and every fortnight subsequently. It was thus possible to chart their growth for more than a year and to compare their progress with that of the other infants in the institution. As far as we are aware, no study of this kind has been attempted in connection with scurvy, although it has long been known that the development of the scorbutic condition is generally accompanied by a cessation of gain in weight. Particular interest would seem to be given this investigation as the subacute type of scurvy must be considered not only the most common form of the disorder, but that which passes most often unrecognized. The opportunity presented itself to probe farther into this question of growth and to determine whether there is not likewise a stunting of the normal increment of the skeleton, an interference with the increase in length. This part of the study was judged to be of greater biologic interest than a mere observation of the effect on weight, as it is well established that infants are particularly tenacious of their impulse to grow in length, and are not readily affected in this respect by nutritional disorders, even such as reach a considerable degree of intensity. As Birk[2] has shown, extreme undernourishment is necessary to bring about stunting. In animals, Aron[3] demonstrated that lack of nutrition led to a decrease of the fat and of the muscle in the body, but that under such conditions the skeleton continued to grow, and the ash content of the body to increase. If, therefore, this function of growth of the body be affected, we must recognize that the metabolism must be profoundly disturbed, and the deficiency of nutritional substances far reaching.

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* Published in Jour. Dis. Children, 1916, 13, p. 152.

[1] Hess, A. F., and Fish, M.: Am. Jour. Dis. Child., December, 1914, viii, 386. Hess, A. F.: Jour. Am. Med. Assn., 1915, lxv, 1003.

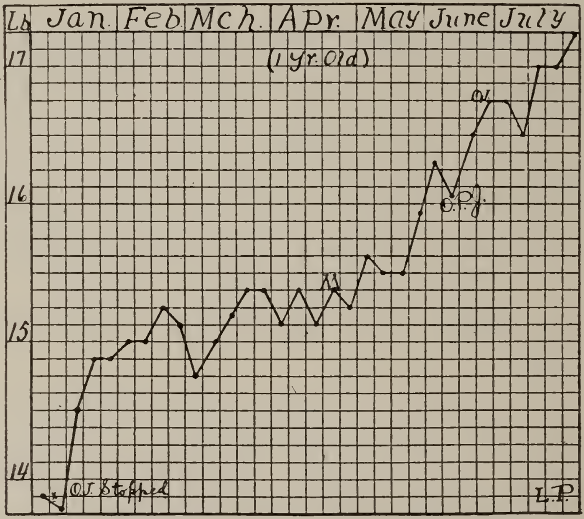

Fig. 1.—Marked gain in weight on orange peel juice and orange juice after lack of gain for three months.

In this and the following figures *O. J.* indicates orange juice; *M.* wheat middlings, and *O. P. J.* orange peel juice.

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2. Birk, W.: Berl. klin. Wchnschr., 1911, No. 27.

3. Aron, H.: Biochemie des Wachstums des Menschen und der hoeheren Tiere, Gustav Fisher, 1913, p. 58.

First, as to growth measured by weight, the various charts (Figs. 1 to 4) accompanying this article show results with regard to this point. It will be noted that although the infants continued to gain for a month or two following the discontinuance of orange juice, a decided flattening of the weight curve gradually set in, an almost constant level being maintained for weeks and months. This stationary period persisted until antiscorbutic food was once more added to the diet, when a sudden rise made itself evident. This gradual cessation of gain and sharp reaction may be stated to have been the rule, as there was but one instance in which an infant continued to gain for months in spite of the lack of antiscorbutic food. It will be seen from these charts that there was no permanent retardation of weight; in other words, the growth impulse of the body remained unimpaired and had been merely in an inactive or quiescent state. When orange juice was given once more, the rate of growth was abnormally great. There was supergrowth. Furthermore, after a period of extended observation of six months or more, it has been found that this increased rate of growth is maintained until the infant reaches a weight normal for its age. These charts seem to require but little elucidation. We may add that orange juice and orange peel juice (an infusion of one ounce of orange peel in two ounces of water), were equally efficacious in bringing about a sharp increase in weight, and that these substances apparently did not lose their potency by being boiled.

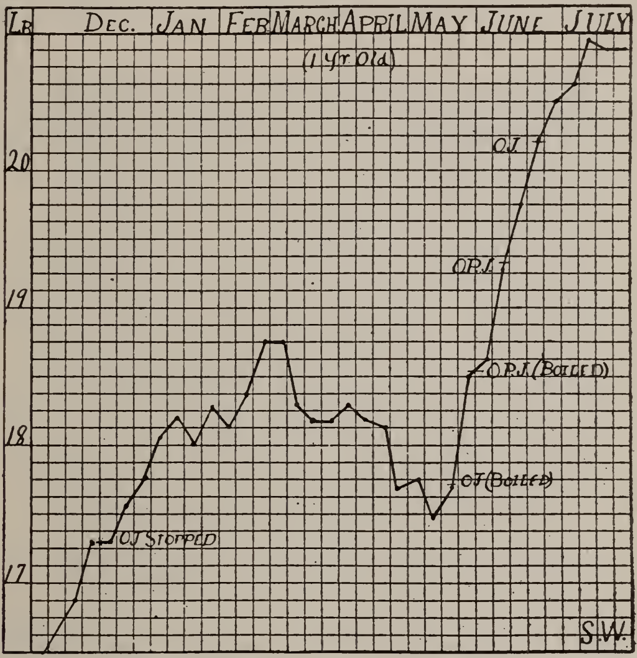

Fig. 2.—Loss of weight checked by boiled orange juice and boiled juice of orange peel.

Two cases should be mentioned especially, as they represent a clinical aspect somewhat different from the others. These infants were under 6 months of age and had never received orange juice. It was possible in these instances to observe for how long a period infants will continue to gain steadily in weight on pasteurized milk before it becomes necessary to add orange juice to their diet, and to judge whether they gradually manifest a lack of gain which can be obviated by giving an antiscorbutic foodstuff. It was found that at about the seventh or eighth month a gradual but definite falling off was evident, and that this deficiency was at once corrected by adding orange juice. Figure 3 represents this condition very clearly. We note here an infant who gained about one-half pound during the months of February, March, April and May, but who in June, when given boiled orange peel juice or orange juice, gained two pounds within a month. There were no other scorbutic signs or symptoms and no loss of appetite during the months of February and March, although the baby was suffering from a progressive form of scurvy. It is probable that this case and a similar one which we encountered are not solitary instances of this kind of reaction, but that many infants fail to gain at this period, the third quarter of the first year of life, for the want of an essential substance in their food, and that unconsciously this deficiency is remedied by adding vegetables and fruit to their dietary. We would therefore urge that antiscorbutics, for instance orange juice, be given infants at an early age. At present, the rule may be said to be to add fruit juices to the dietary at about the sixth month. This period has been chosen as the proper one, probably because scurvy rarely develops during the first half year of life. However, when we reflect that this time incidence is due to the fact that the infant is protected for the first few months of life by the supply of antiscorbutic material which it has received from the mother, and that there must be a constant negative balance of these essential substances dating from the earliest beginning of artificial feeding, it would seem as if a corrective dietary, that is to say, an antiscorbutic food, should be given as soon as practicable. There is no reason that I know of why an infant should not receive orange juice when it is a month old, and, as we have seen, there are strong arguments in favor of such a procedure.

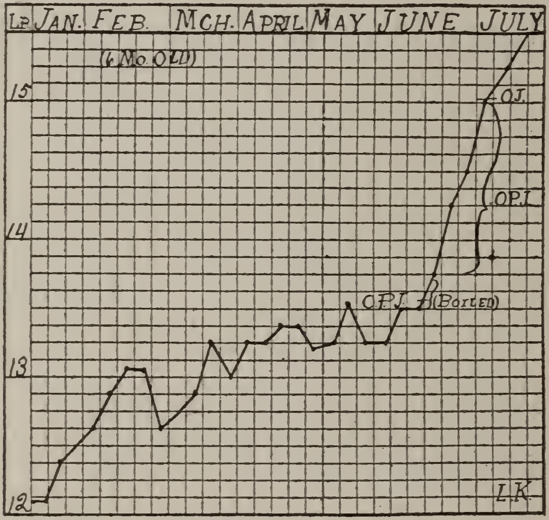

Fig. 3.—Latent scurvy in young infant who had never received orange juice. Scorbutic nature of growth disorder proved by sharp rise in weight on addition of antiscorbutic food to the dietary of pasteurized milk.

It may be enunciated as a rule, embracing a few exceptions, that the development of the scorbutic state is generally accompanied by a failure to gain in weight. Under certain conditions, however, the weight may follow a perfectly normal course during the entire period. This observation has, from time to time, been made by others, but has received no particular consideration. It may be well therefore to cite in some detail a case of this kind, and discuss one cause of this apparently paradoxical course of events. Figure 4 illustrates this clinical paradox. The baby to whom it refers was admitted to the institution in January and was stated to be only four months of age. As a matter of fact, as we learned later, he was seven and a half months old. Toward the end of February, when he had been in the institution two months, in spite of constant and normal gain in weight, to our surprise he manifested unmistakable signs of scurvy—peridental hemorrhage over the upper incisor teeth, which were erupting, and tenderness of the lower ends of the femora. The scorbutic nature of these signs was substantiated by their prompt subsidence on the administration of orange juice.

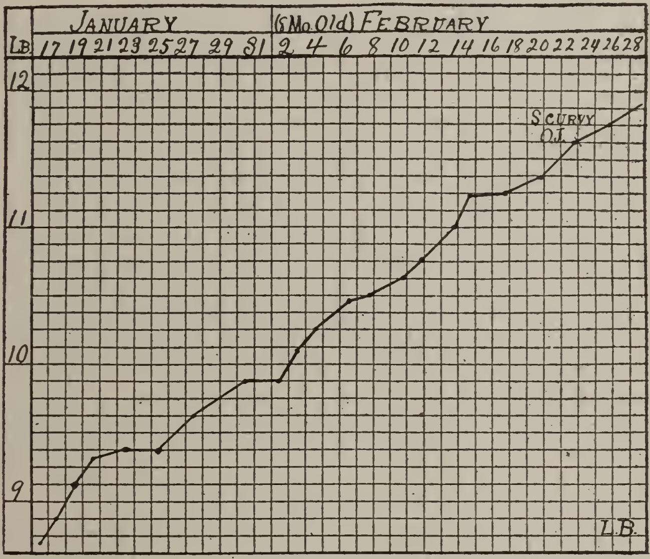

Fig. 4.—Development of scurvy in spite of normal gain in weight in a baby who had been underfed since birth.

How is this phenomenon to be explained, normal growth during the development of scurvy, one of the classical nutritional disorders of infancy? The solution is to be found in a careful consideration of the previous diet of the baby. On investigation we found that it was born on June 5, 1915, at term, weighing six pounds, but was never nursed. For the first two weeks it received an indefinite formula prepared by the mother. From June 21 to August 19, that is, for a period of about two months, it was given a formula composed of one-fourth milk, three-fourths water, and 1.5 per cent. sugar, two ounces every three hours. In other words, it was greatly underfed for these two months. In August it weighed 8 pounds, 5 ounces. From August 19 to 27 the formula was 30 ounces of water, 12 ounces of milk, and about 1 per cent. sugar, four ounces being fed every three hours. This resulted in loss of weight, the baby weighing 7 pounds, 15 ounces. On September 7, the formula was made one-half milk, one-half water and 1 per cent. sugar, four ounces every three hours. The underfeeding continued. On December 5, it was transferred to a hospital, where it was given a malt soup preparation for one month. It developed gastro-enteritis on January 17. As the chart shows, the child was admitted to the Hebrew Infant Asylum, weighing about 8¾ pounds at the age of 7½ months.

If we consider the feeding of this infant, we realize that it had been almost continuously undernourished, receiving an insufficient amount of fats and carbohydrates at almost all times. At the asylum the baby was put on Schloss milk, a food containing about 4 per cent. fat and about 6 or 7 per cent. carbohydrates, seven feedings of 4 ounces in the twenty-four hours. On this it remained and, as will be seen, made steady gains until it developed scurvy toward the end of February.

This baby must be regarded as having been partially starved throughout the period following birth, with the result that on receiving a diet rich in fats and carbohydrates, foodstuffs which bring about a marked increase in weight, the reaction of the tissues was necessarily prompt and prolonged. It explains this type of case, showing that if an infant has received insufficient food, we can compel growth simply by increasing the caloric value of the dietary, notwithstanding the fact that scurvy is developing day by day. It also brings out the salient fact that more than a single cause exists leading to a repression of growth, and that therefore lack of growth must be differentiated if it is to form the basis of study. A deficiency of scurvy vitamins is one cause, lack of sufficient or adequate food is another, and, no doubt, there are still other inciting factors. Whether or not growth occurs, and to what extent, depends on the resultant stimulation which can be brought about by these various impulses. In this instance the primary growth impulse which follows a diet containing a sufficient number of calories had been held in abeyance for so many months through starvation, that when it was once more stimulated to full activity by a liberal diet, it was able to overcome the growth repression which ordinarily accompanies the development of the scorbutic condition.[4]

The foregoing illustration must make it clear that growth does not play an essential or elemental part in the constitution of infantile scurvy. It must likewise render it evident that this study cannot be regarded as concerned with growth in general, but only with the effect on growth of infantile scurvy as one of the various disorders which may affect this function. That growth may be so influenced is well known to clinicians and has been shown admirably in relation to animals in the recent investigations of Osborne and Mendel, of McCollum and others, who demonstrated that growth was retarded or stimulated at will by means of diet. The results of these workers cannot, however, be considered as having any bearing on scurvy, for scurvy and growth stunting are by no means identical, either in animals or in infants. It would seem that these remarks are timely in view of a recently published study by McCollum and Davis,[5] in which they show that the growth factor in milk is closely linked to its casein content, and preface their report by drawing an analogy to infantile scurvy, although apparently none of the animals showed any signs of a scorbutic condition.

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[4] This phenomenon probably also holds good for scurvy in the adult. A scorbutic condition may result from a lack of fresh food; but if the person has been markedly underfed, there may be a gain in weight coincident with the development of the scorbutic condition, provided liberal diet is given, including plenty of carbohydrate and fat.

[5] McCollum, E. V., and Davis, M.: Jour. Biol. Chem., November, 1915, p. 247.

Looking at this question from the reverse point of view, one sees clearly that factors which stimulate growth are not necessarily antiscorbutic. For example, McCollum and Davis[6] showed that butter fat, even after it had been heated to a high degree, was able to induce growth, and Osborne and Mendel[7] showed that this fat can be subjected to steam and not lose its growth producing power. It is nevertheless evident that butter fat in pasteurized milk does not possess sufficient antiscorbutic properties to prevent the development of scurvy. Substances such as lysin and tryptophan, which possess marked growth promoting power, and which were present in considerable amount in the diet of our cases, were unable also to make up for the dietary deficiency and bring about growth. Furthermore, cod liver oil has been found to possess growth-promoting qualities of a high degree, but there is no question that this substance, as has been shown elsewhere[1] is incapable of preventing the development or of accomplishing the cure of scurvy. Before reporting additional observations as to the influence of scurvy on growth, let me add that all of the infants under consideration obtained plenty of milk, and all the older ones received cereal in addition. Particular attention was given to their obtaining a sufficient quantity, so that the factor of an insufficient diet might not enter into the question. To this end, when orange juice was discontinued, more cereal was given or the strength of the milk mixture was increased in many instances. In those cases in which there was loss of appetite, particular effort was made to have the infants take the full quantity of food, and, although the total amount consumed in many cases was not quite as much as when they were well, it nevertheless equaled that which many infants in the institution consume and on which they continue to grow.

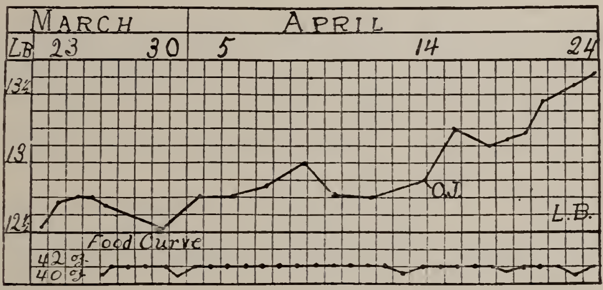

Fig. 5.—Same case as preceding. Detailed chart showing marked gain following the addition of an antiscorbutic to the diet, although amount of food remained the same.

Gain of 5 ounces in three weeks before, and 12 ounces in ten days after orange juice was given.

There is no doubt that considerable of the supergrowth which so clearly follows the giving of orange juice or its equivalent is due to an increased consumption of food. However, it would be a mistake to consider that such is entirely the case. We have reproduced two charts to illustrate this point. The first (Fig. 5) is a daily weight chart of an infant with mild

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6. McCollum, E. V., and Davis, M.: Jour. Biol. Chem., 1913, xv, 167.
7. Osborne, T. B., and Mendel, L. F.: Jour. Biol. Chem., May, 1915, p. 381.

scurvy, and shows the period preceding as well as that following the giving of orange juice, together with the food intake (Schloss milk). Although it will be seen that there was no change in the amount of milk taken before and after giving orange juice, there was a marked difference in the gain; the infant increased only 5 ounces in weight during the three weeks in which it did not obtain orange juice, and 12 ounces in ten days following its addition to the diet. Figure 6 shows another aspect of this question. It demonstrates that lack of food is not responsible for the period of gradual growth stagnation into which infants enter when fed entirely on pasteurized milk without receiving an antiscorbutic also. Here we note the gradual inception of this stationary phase after a period of gain, in spite of the fact that the intake of food remained undiminished. It is evident, therefore, that neither the lack of gain in the course of the development of infantile scurvy, nor the increase of weight coincident with its cure, can be considered to be dependent on the caloric value of the dietary.

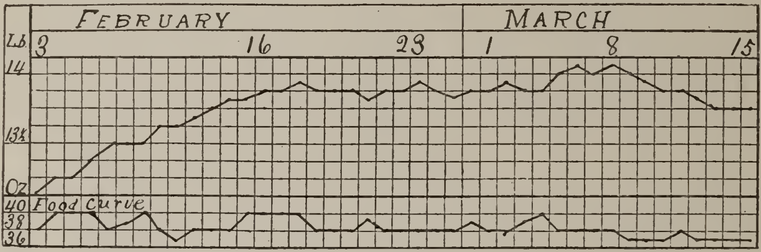

Fig. 6.—Section of a weight chart showing daily weighings during the period when gain still continued and when stationary plane was reached.

Note that the same amount of food was taken daily during the first two weeks of February, when the infant gained 12 ounces, as from the middle of February to the middle of March, when there was no gain whatever.

This baby had latent scurvy and responded promptly to orange juice.

As has been mentioned above, a study of the growth in length was also carried out, the measurements being taken fortnightly for over a year.

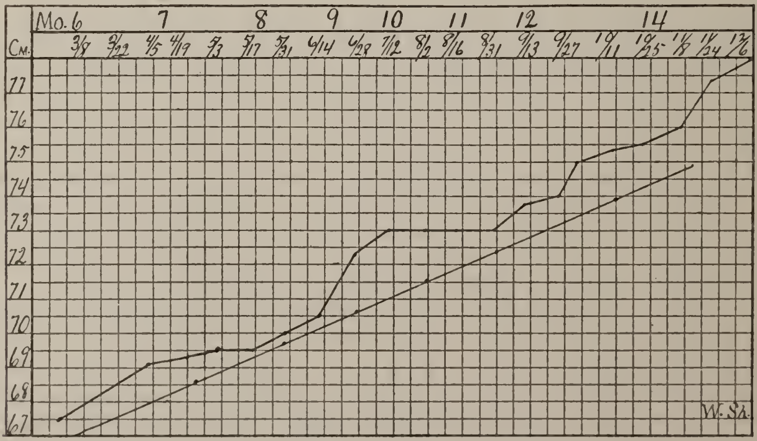

Fig. 7.—Growth in length from the sixth to the fifteenth month of healthy baby receiving orange juice. The lower line represents the average growth.

In order to furnish a basis for comparison, the growth of ten normal infants who received orange juice, but otherwise the same diet, was followed at the same time. The charts which we reproduce clearly bring out the distinction between the two groups of cases. Figures 7, 8 and 9 illustrate the growth of the normal infants, and constitute a standard by which to judge the others. They portray a consistent, although slightly irregular, growth month by month. Quite in contrast to these curves are those depicted in Figures 10, 11 and 12, representing three infants who did not receive orange juice from January to May.

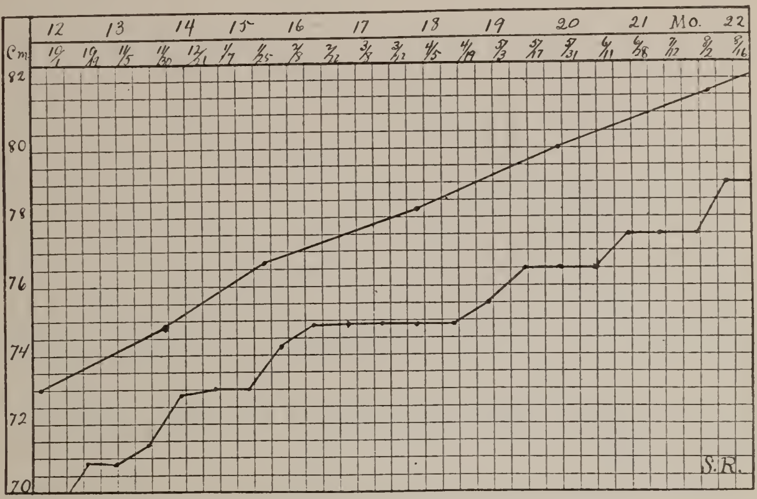

Fig. 8.—Growth in length from the twelfth to the twenty-second month of a very small, healthy baby receiving orange juice.

Figure 11, which represents growth in length complements Figure 3, representing growth in weight, and illustrates the case of a young baby

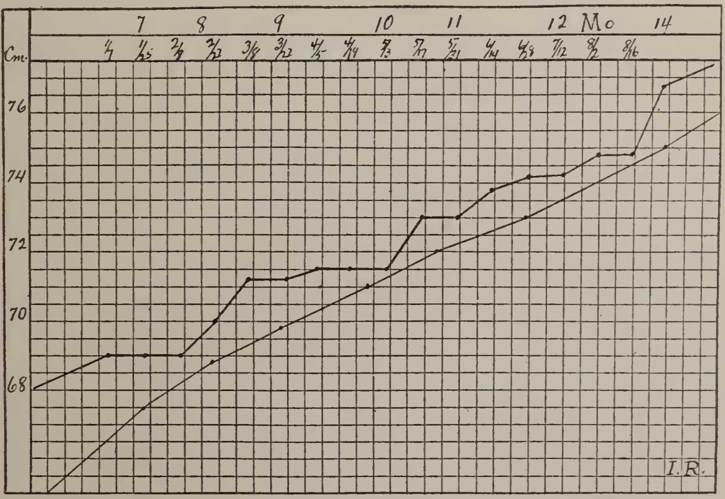

Fig. 9.—Growth in length from the seventh to the fourteenth month of a large healthy baby receiving orange juice.

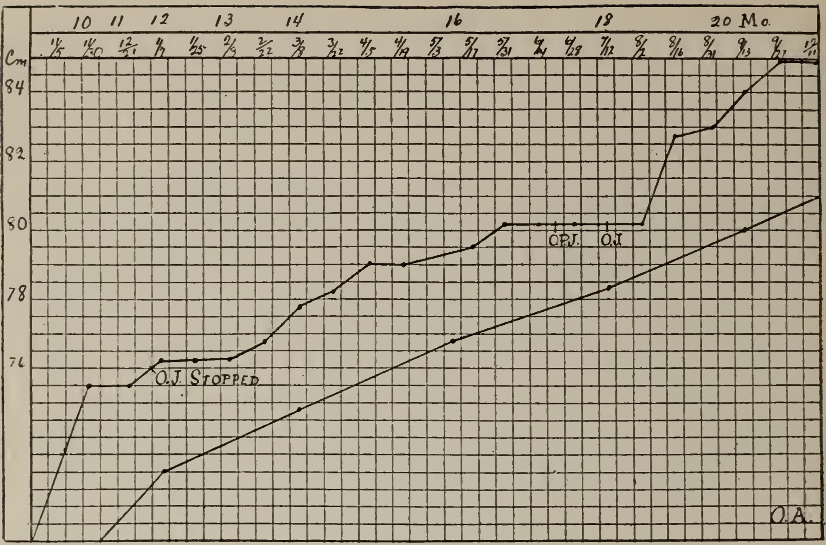

Fig. 10.—Growth in length for a period of five months during which no orange juice was given, compared with subsequent months when it was again added to the diet.

who had never received orange juice, and developed a latent or rudimentary form of scurvy when about eight months of age. These three cases, when contrasted with the normals, leave no question that the scorbutic condition has a decided effect on growth in length as well as in weight, and that

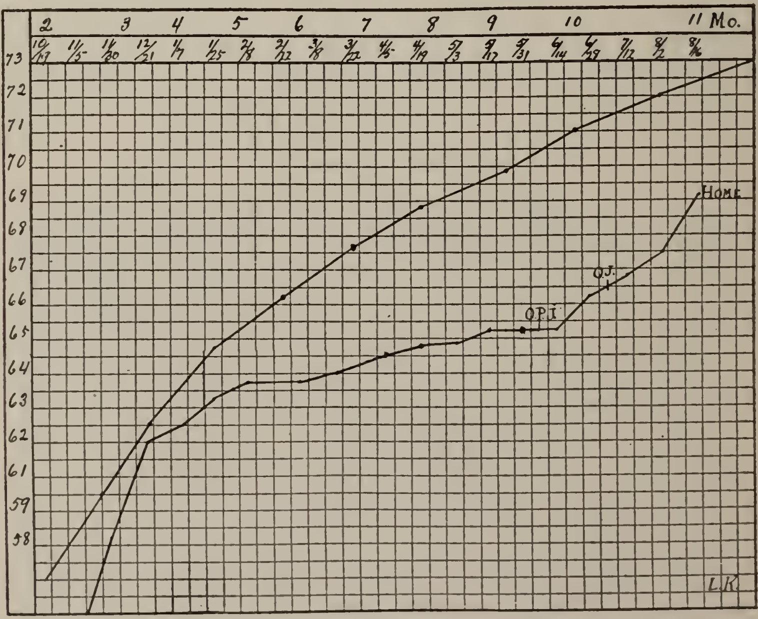

Fig. 11.—Length chart of young infant (illustrated in Fig. 3) who had never received orange juice Marked reaction in growth in length when an antiscorbutic was added to the diet.

this impulse remains unimpaired in both respects and capable of quick response when the essential foodstuff is furnished.

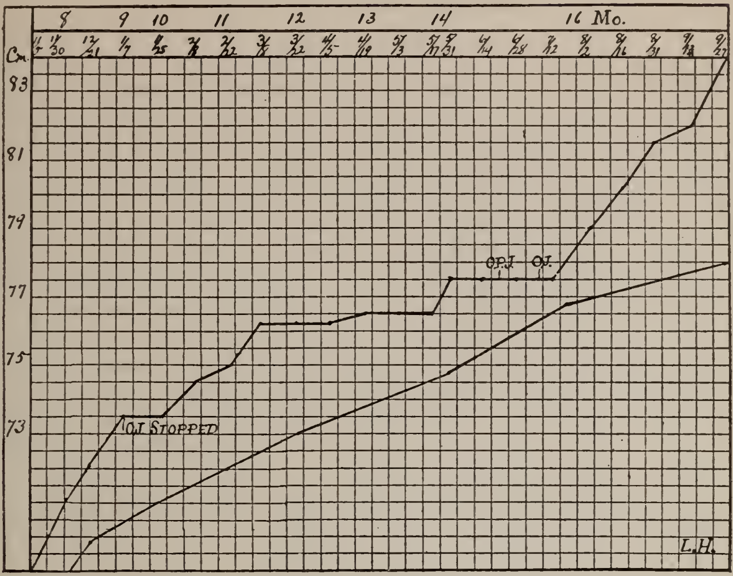

Fig. 12.—Showing retardation of growth in length during the period when no orange juice was given, and supergrowth when it was given once more.

CONCLUSIONS

Although pasteurized milk is to be recommended on account of the security which it affords against infection, we should realize that it is an incomplete food. Unless an antiscorbutic, such as orange juice, the juice of orange peel, or potato water is added, infants will develop scurvy on this diet. This form of scurvy takes some months to develop and may be termed subacute. It must be considered not only the most common form of this disorder, but the one which passes most often unrecognized. In order to guard against it, infants fed exclusively on a diet of pasteurized milk should be given antiscorbutics far earlier than is at present the custom, even as early as at the end of the first month of life.

In the course of the development of infantile scurvy, growth, both in weight and in length, is markedly affected. Under this condition, weight ceases to increase, and a stationary plane is maintained for weeks or for months. There is a quick response, however, on the administration of orange juice or its equivalent, indeed supergrowth is thereupon frequently manifested. If, however, an infant has been underfed, an increase in weight may continue throughout the development of the scorbutic condition. Although the caloric value of the food remains unchanged, cessation of growth, as well as marked increase in growth, may be manifested, depending merely on the withholding or the addition of essential foodstuffs to the diet.

Measurements showed that growth in length is also retarded during the protracted development of infantile scurvy. This is of greater biologic interest, as simple malnutrition does not usually affect this function in the infant. In this particular, supergrowth also follows the addition of the essential foodstuff, showing that the growth impulse has remained uninjured and has been merely held in abeyance.

A FURTHER REPORT ON THROMBOPLASTIN SOLUTION AS A HEMOSTATIC.*

Alfred F. Hess.

Somewhat over a year ago in a preliminary report on "Tissue Extract as a Hemostatic,"[1] it was stated that a very efficient hemostatic could be prepared from brain tissue and that this preparation, which was termed "thromboplastin," had been found serviceable for local use in hemorrhage, especially in bleeding associated with true hemophilia. This substance is a solution and a fine suspension of ox brain in normal salt solution with 0.3 per cent. of tricresol added in order to preserve it. Some months later a paper was published by Cronin[2] stating that he had made use of "thromboplastin" in a routine way in over 2,000 tonsil and adenoid operations which were performed in the clinics of the New York Department of Health and that he found it of decided value in decreasing bleeding and the incidence of undue hemorrhage. For about a year the Research Laboratory has distributed thromboplastin solution to physicians and has collected reports on the results obtained. During this period I have had many occasions to make further trials of its clinical value and have also carried out numerous laboratory tests to determine more fully its various properties. It is the sum of these twofold experiences which forms the basis of this second report.

Thromboplastin solution has been supplied to several of the maternity hospitals of the city where it has been employed locally in cases of melena neonatorum, in bleeding from the cord, skin, mouth, vagina, etc. It has also been used by physicians in these institutions as a dressing where there was undue hemorrhage following circumcision. The two cases following illustrate its application in melena:

Case 1.—April 18, 1916, baby 1 week old, seen at the Bellevue School for Midwives, on the service of Dr. Austin Flint, showed numerous cutaneous hemorrhages, as well as some submucous hemorrhages in the mouth at the junction of the hard and soft palate. The main site of bleeding was the cord. There had been some bleeding from the bowel; also moderate jaundice. Thromboplastin solution was applied to the cord; the bleeding was checked and did not recur.

A case which is of particular interest on account of the persistence of the bleeding and the fact that numerous other hemostatics had been previously applied without result was the following:

Case 2.—Jan. 15, 1916, a 5-day-old baby was seen on Dr. Edwin Cragin's service at the Sloane Maternity Hospital. The frenum of the tongue had been incised two days previously, and since this time hemorrhage had persisted. When seen, the infant presented some subcutaneous ecchymoses and a small hematoma of the scalp, and was evidently suffering from loss of blood. Various measures had been tried to check the bleeding. Human serum and whole blood had been injected subcutaneously. Locally silver nitrate had been used, tannic acid applied assiduously for a period of two hours, and clamping had been attempted. The bleeding persisted for two days in spite of all these efforts. Thromboplastin solution was applied locally with partial success. Following this a few cubic centimeters of the preparation (which had been brought to the boiling point) were injected into the site of the hemorrhage at the root of the tongue. The bleeding stopped at once and did not recur.

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* Published in Jour. Am. Med. Ass., 1916, 67, p. 1717.

[1] Hess, A. F.: Tissue Extract as a Hemostatic, Journal A. M. A., April 24, 1915, p. 1396.

[2] Cronin, J. J.: Thromboplastin (Tissue Extract) as a Hemostatic, The Journal A. M. A., Feb. 22, 1916, p. 557.

This case raises the question as to the most effective method of applying thromboplastin solution. In this connection it may be stated that, as in the instance just cited, where topical applications do not check the bleeding, some of the solution should be boiled and if possible injected into the site of the bleeding. This method of application is especially suitable in bleeding from the gum, which frequently offers great difficulties following the extraction of a tooth. The following is a case in point:

Case 3.—Oct. 5, 1915, a young man with splenomyelogenous leukemia was seen at Beth Israel Hospital. A tooth had been extracted five days previously, and since then there had been almost continuous bleeding. Serum had been given subcutaneously; serum, human blood, epinephrin, coagulin and emetin had been applied locally without avail. Thromboplastin solution was then applied locally, but as it did not check the steady oozing, some was heated to the boiling point and injected into the gum. The bleeding promptly ceased.

In discussing the method of applying thromboplastin solution, emphasis should be laid on the necessity of having it come into direct contact with the bleeding point; if soft clots have formed, they should be removed from the wound. Furthermore, although a cursory application in many instances will suffice to hasten coagulation,when such does not prove to be the case the hemostatic should be held in close contact with the bleeding surface for some minutes. From time to time cases have been encountered in which attention to this detail has checked hemorrhage which had seemed uncontrollable. For example:

Case 4.—Nov. 1, 1915. Bleeding in young adult following tonsillectomy. Tonsils had been excised twenty-four hours previously and the patient had bled ever since. He looked pale; the red blood count was two and one half million. Emetin had been applied locally, as well as pressure. Salt solution had been given intravenously. Thromboplastin solution was applied locally and was thought to be of no avail; applications were being made every minute or two at the time the patient was seen. When, however, the swab saturated with the solution was left in contact with the bleeding surface for from ten to fifteen minutes (so that a firm clot would have time to form), the bleeding stopped permanently.

It seems unnecessary to cite further instances of the efficacy of this hemostatic when applied locally. In a very few cases it has failed although the reason has not been clear.[3] As was emphasized in the previous report, it must be regarded almost as a specific in the bleeding of true hemophilia, a disease characterized by a prolonged coagulation time. This is as we should expect when we consider the remarkable coagulative power of this preparation when added directly to blood or to plasma. Further clinical experiences have served only to strengthen our reliance on thromboplastin solution in this hemorrhagic condition, so that we believe that those who suffer from this disease would do well to have a preparation of tissue juice at hand in case of emergency. It is the general experience that these "bleeders" frequently die, not from a sudden loss of blood, but from a persistent oozing from the tongue or mouth which gradually drains their vitality. Hemorrhages of this type yield promptly to thromboplastin.

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[3] These intractable hemorrhages have almost invariably followed circumcision, and all local measures have failed, so that transfusion had to be resorted to.

There have been many inquiries as to the advisability of using this preparation in gastric or intestinal hemorrhage. It is very difficult to judge of the success of any therapy in cases of this kind, and thromboplastin will require trial in many more such cases before a definite opinion can be formulated. It has hardly seemed that this preparation would be efficacious in bleeding from the ileum or from the large intestine, as much of it would be digested by the proteolytic ferments and destroyed by the time it reached this part of the intestinal tract. However, we may find that if large amounts are taken by mouth or if it is given in a form which will protect it from disintegration, or, perhaps, if it is poured directly into the intestine through a duodenal tube, it will prove of value in bleeding from the lower bowel. As far as we are aware, it is nontoxic when given by mouth; 60 c.c. have been given in the course of twenty-four hours and have produced no untoward effects; we should not hesitate to give double this quantity. In gastric or duodenal hemorrhage it has seemed of value and should certainly be given an extended trial. It may also be used locally in rectal bleeding by means of a proctoscope. The following four cases, Case 5, gastric hemorrhage from ulcer, Case 6, gastric hemorrhage in hemophilia, Case 7, duodenal hemorrhage in an infant, and Case 8, rectal hemorrhage in a hemophiliac, illustrate these several conditions:

Case 5.—April 26, 1916, man, admitted to Beth Israel Hospital, had severe gastric hemorrhage. Thirty c.c. of thromboplastin solution were given in 6 ounces of water and three 20 c.c. doses diluted in 4 ounces of water during the following day. There was no further hemorrhage.

Case 6.—Jan. 3, 1916, hemophiliac boy who had been under observation for several years vomited a considerable quantity of blood. He was sent to the hospital, where he vomited blood twice. He was then given 10 c.c. of thromboplastin solution diluted in 2 ounces of water; this dose was repeated in three hours. There was no further hematemesis.

Case 7.—April 17, 1916, baby born the previous day at Bellevue Hospital, in Dr. Austin Flint's service, that evening vomited blood at 5:30, and twice again before 10 o'clock. Thromboplastin solution was given by mouth, 2 c.c. in a tablespoonful of water. There was no vomiting for twenty-four hours. Hematemesis recurred, with bloody stools, and death followed.

Case 8.—Dec. 28, 1916. Typical hemophiliac (brother of the patient in Case 6). Blood had been noted in the stool since December 6 and had increased until five days previously, when pure blood was passed. He was sent to the hospital where protcoscopic examination showed an ulcer in the rectum. Thromboplastin solution was applied on strips of gauze, and bleeding ceased. This treatment was repeated on the following day. There was no further bleeding while the patient was in the hospital.

It may be of advantage to note a few other conditions in which our records show that thromboplastin solution has been used successfully. In this connection may be mentioned the nasal hemorrhage associated with toxic conditions, more especially diphtheria, for which thromboplastin has been employed extensively at the Willard Parker Hospital for Contagious Diseases; nasal hemorrhage complicating the primary blood diseases, for example, in acute leukemia; at operation for hepatic bleeding; in bleeding from the urinary bladder; in operations involving the spinal cord, in prostatic operations and in bronchoscopy in connection with the removal of diphtheria membrane or old foreign bodies with bleeding granulation tissue.

As my preliminary paper contained nothing whatsoever regarding the action of thromboplastin solution in vitro, it may be well to report some tests showing its action on plasma and to illustrate how this activity is affected by various manipulations, boiling, dilution, centrifugation, etc. Table 1 demonstrates the marked clotting power of 1 drop of thromboplastin solution on 5 drops of plasma; it shows also that a 1 per cent. dilution of this preparation acts almost as well as the undiluted fluid. This has been observed frequently in similar tests in which it required a dilution even greater than 1 per cent. to diminish the coagulative efficiency of the thromboplastin. The phenemenon is probably explained by the fact that in the undiluted state thromboplastin possesses a great excess of coagulative power. It may be added that it has been found to possess some potency even when diluted 5,000 times and that I drop of a 1:500 solution has lessened the coagulation time of plasma by one half. Table 1 also ilustrates that a cursory boiling serves to weaken the preparation only slightly and does not radically impair its power.[4] It may be pasteurized without affecting its potency (Table 4).

TABLE 1.

CLOTTING EFFICIENCY OF THROMBOPLASTIN SOLUTION.

Human Plasma.	Thromboplastin Solution Unboiled		Thromboplastin Solution Boiled (One Minute)	
	Strength of Thromboplastin Solution Added,* per cent.	Coagulation Time of Plasma. Minutes.	Strength of Thromboplastin Solution Added, per cent.	Coagulation Time of Plasma. Minutes.
Test A, with plasma having coagulation time of ten minutes...............	100.0	1	100.0	2.5
	50.0	1	50.0	2.5
	10.0	1	25.0	2.5
	1.0	2	1.0	4.0
	0.5	4	0.5	6.0
Test B, with centrifugalized plasma having coagulation time of twenty-two minutes.....................	10.0	3	10.0	6.0
	1.0	5	1.0	8.0
Test C, with plasma having coagulation time of thirteen minutes...........	100.0	1	100.0	1.5

* In all tests 1 drop of thromboplastin solution, 5 drops of oxalated plasma, and an optimal amount of calcium chlorid solution were used.

When thromboplastin solution is filtered, it becomes a clear, transparent fluid which possesses considerable coagulative power. Table 2 shows the efficiency of the filtrate of a 2 per cent. dilution; it shows likewise that this weak filtrate even after being boiled is able to cut in half the coagulation time of plasma. The fact is also brought out in the table that coagulative potency resides in the supernatant fluid obtained after centrifugation. This shows that we are dealing with a substance which is active in a clear solution as well as in turbid suspension.

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[4] Occasionally, as may be seen in Table 2, a brief boiling enhances rather than weakens the efficiency of the preparation.

Table 3 is included to demonstrate that the coagulative potency of the gray and of the white matter of the brain is practically the same. In view of the results of these and of similar tests, we have made use of the brain in its entirety in preparing thromboplastin solution.

TABLE 2.

EFFECT OF FILTRATION, CENTRIFUGATION, ETC., ON POTENCY OF THROMBOPLASTIN SOLUTION.

Preparation of Thromboplastin Solution (2 per cent. Dilution).	Drops of Thromboplastin	Coagulation Time (5 Drops of Old Oxalated Plasma) Minutes.
Control plasma (no thromboplastin)	..	24
Untreated solution	1	10
	2	8
	3	8
	4	6
Boiled (1 minute)	1	8
	2	6
	3	6
	4	6
Filtered	1	10
	2	8
	3	8
	4	6
Centrifugalized (supernatant fluid)	1	10
	2	8
	3	8
	4	6
Filtered and boiled	1	14
	2	12
	3	10
	4	8
Centrifugalized and boiled	1	14
	2	12
	3	10
	4	8

In a similar test, plasma which coagulated in thirteen minutes clotted in one minute on addition of 1 drop of undiluted thromboplastin solution; in one and one half minutes when the solution had been boiled; in three minntes when the supernatant centrifugalized fluid was used, and in two minutes when this fluid had been previously brought to the boiling point.

TABLE 3.

COMPARATIVE TESTS OF ACTIVITY OF THROMBOPLASTIN SOLUTION PREPARED FROM BRAIN CORTEX AND FROM MEDULLA.

One per cent. Thromboplastin Solution (1 drop).	Coagulation Time Old Sheep Plasma, Minutes.	Coagulation Time Old Horse Plasma, Minutes.
Control plasma (no thromboplastin)	22	14
Medulla	3	2
Cortex	3	3
Medulla (solution boiled)	6	6
Cortex (solution boiled)	4	5

Thromboplastin may be desiccated at a low temperature and then used as a hemostatic. We have prepared a powder of this kind diluted with a large quantity of lactose and preserved by means of boric acid. This powder was able to hasten coagulation in vivo as well as in vitro and was found of clinical value under certain conditions. Neither boric acid nor the tricresol made use of in the fluid preparation lessened the potency of thromboplastin solution.

We have also made considerable use of weak dilutions of thromboplastin solution for intravenous, subcutaneous and intramuscular inoculation where the site of the bleeding was inaccessible to local treatment.

TABLE 4.

EFFECT OF HEATING THROMBOPLASTIN SOLUTION FOR ONE HOUR AT 55 C.

Preparation of Thromboplastin Solution.	Drops of Thromboplastin.	Coagulation Time (5 Drops of Old Oxalated Plasma), Minutes.
Control plasma (no thromboplastin)	..	20
Thromboplastin solution, unheated	1	2
	2	1
	3	1
Thromboplastin solution, heated	1	2
	2	1
	3	1
Thromboplastin solution, heated and centrifugalized	1	2
	2	2
	3	1

Although it is too early to report on this aspect of the subject, it may be stated that when proper dilutions are employed this mode of therapy is without danger.

There is quite another aspect of this subject which is of interest. In the course of the use of thromboplastin solution as a hemostatic, it was noted that it stimulated the healing of wounds and of raw surfaces. This was first called to our attention by a patient suffering from hereditary telangiectasis of the nasal mucous membranes, who, in addition to frequent attacks of epistaxis, suffered from superficial ulcers in the anterior nares. He noted that the thromboplastin solution not only checked the hemorrhages, but healed these ulcers which had been of long standing. Prompted by this experience, thromboplastin solution has been employed for the past six months or more as a dressing of wounds and of ulcers of long duration. These observations have been carried out for the most part at the Central and Neurological Hospital of the Department of Public Charities, where inmates remain for months or years and therefore can be observed for long periods. This work was kindly undertaken by Dr. Harold Neuhof who makes the following report:

Between eighteen and twenty cases have been treated with thromboplastin solution for a sufficiently long period to gage the results. Twelve were chronic ulcers of the leg, eleven varicose and one syphilitic. All showed that the fluid thromboplastin is an active stimulant, aiding healing. Several ulcers of long standing were healed completely; one recurred subsequently, to heal promptly with further treatment. One case of broken down laparotomy wound responded well to the fluid, also a sluggish ulcer of the neck following operation for carbuncle in a diabetic patient. A case of ulcer in the axilla following burns did very well as did an ulceration about the umbilicus following infection. In none of the cases was thromboplastin solution used until it was evident that we were dealing with sluggish, chronic lesions that did not respond to the usual treatment. Leg ulcers of long standing (Central Neurological Service) cleaned up very nicely, but such lesions when very extensive and of many years' standing, did not progress to healing. The lesions were put in such condition, however, that further (operative) measures could be considered advisable, whereas heretofore they were out of the question.[5]

In this report a fluid thromboplastin is mentioned in contradistinction to an ointment which was also used for healing purposes. To make the ointment, one part of brain substance was ground up with three parts of lanolin which had been heated to a fluid state; 3 per cent. boric acid was added as a preservative. It was found, however, by Dr. Neuhof as well as by others that the ointment applied to raw surfaces did not bring about as prompt response as did the fluid preparation. When used as a local application in eczema, especially in some cases in infants, the ointment seemed to show a marked curative effect. With regard to the fluid thromboplastin it may be added that we have received a favorable report on its use as a drip in conjunction with the application of skin grafts to wound surfaces.

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[5] In treating ulcers, the surrounding skin should be protected from irritation by petrolatum or some bland ointment. The beneficial effect of thromboplastin is possibly due to its ability to remove infected or unclean sloughs from the surface of the ulcers, possibly in part to the direct effect on the tissues with which it comes into contact.

Conclusions.

Tissue juice made from brain (thromboplastin solution) has proved itself of practical value in controlling hemorrhage wherever it can reach the site of bleeding. In cases of true hemophilia it may be regarded almost as a specific hemostatic. It is to be recommended for local use in the bleeding of the new-born, in nasal hemorrhage and in the parenchymatous bleeding associated with various operations, etc. Where local applications fail, it should be injected into the site of hemorrhage as in bleeding from the gums following tooth extraction. This method can readily be resorted to as thromboplastin solution loses but little of its potency as the result of dilution and cursory boiling.

Further clinical experience is necessary before its value can be determined as a hemostatic in connection with hemorrhage of the gastro-intestinal tract. However, it is innocuous when given by mouth in considerable dosage and would seem to be indicated in bleeding from the stomach and from the upper intestine.

In addition to its hemostatic action, this tissue extract has been found to possess healing properties, actively stimulating granulation tissue and hastening epithelization. It is therefore applicable as a dressing for torpid ulcers and for sluggish wounds.

PROMPT MACROSCOPIC AGGLUTINATION IN THE DIAGNOSIS OF GLANDERS.*

OLGA R. POVITZKY.

The agglutination test for the diagnosis of acute cases of glanders is conceded by all workers to be of great value. Miessner (1) and others have shown that agglutinins in glanders are generally at their height between the fifth and eleventh day of the infection, after which time they begin to decline. At the end of two months they may fall even below 1:500. This test, if used alone, would fail to detect chronic cases and it should, therefore, be used as an adjunct to the complement fixation[1] and ophthlamic mallein[2] tests. No one test can be depended on alone as each one has its peculiar value in certain stages of the disease. If all these tests are combined, very few cases of glanders can escape detection. Even though a case may appear to be negative by one or two of the methods, it may be found positive by the third.

The great objection to the employment of the macroscopic agglutination method used heretofore in the routine diagnosis of glanders, is the length of time required for the appearance of a reaction. While some tests can be read at the end of twenty-four hours, others do not show definite agglutination within forty-eight or seventy-two hours or even longer. This drawback led to the centrifuge method for which Muller (2) claims priority. By it the process of the agglutination reaction is shortened considerably. Miessner (1) also Pfeiler (3) employed this method claiming it to be a great success.

According to this method, the test fluid is prepared in the same way as in the old method—namely, a forty-eight hour growth of a suitable strain of *B. mallei* is suspended in salt solution and heated at 60°C. for two hours. It is then diluted to a certain density with 0.5 per cent. carbolic acid. The tubes are set up in the ordinary way, each tube containing 2 to 3 c.c. of the titrated test fluid with varying quantities of serum to make the final dilution, 1:400, 1:500, 1:800, 1:1000, etc. After one hour incubation at 37°C. the tubes are centrifuged for ten minutes, after which they are allowed to stand at room temperature for one and one-half hours. The tests are read at the end of that time. The appearance of an irregular veil like clumping at the bottom of the tube with clearing of the upper portion is considered an agglutination, while a dense white precipitate with a cloudiness of the upper part is considered a negative reaction.

Though the centrifuge method possesses great advantages over the old method in point of time, it is difficult always to separate an agglutination from a sedimentation and the result is not always readable. Thus, Anthony and Grund (4) found this test unreadable in 12 per cent. of the cases tested, even after an incubation of twenty-four hours.

In order to eliminate the shortcomings of the former methods, I have worked out a modification by which an agglutination reaction, clear cut in appearance, can be obtained in less than two hours with positive sera in dilutions up to 1:2000 or higher.

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[1] Specific amboceptors for the complement fixation test may be demonstrated seven to ten days after infection and they remain during the course of the disease (6).

[2] The ophthalmic mallein test is reliable twenty-one days after infection while the subcutaneous mallein test may be relied upon for diagnosis fifteen days after infection (6).

The strain of *B. mallei* used was one called B. M. 5, isolated at this laboratory about five years ago from a case of human glanders. Fourteen other strains from various sources (chiefly horses) were tested also, but none have given invariably the prompt and clear cut reaction that B. M. 5 does. The specificity of this strain was ascertained by testing it with a number of syphilitic, typhoid, streptococcus and pneumococcus sera; also with a number of sera from horses with equisepticus. One of the latter sera gave a positive reaction but the possibility of glanders could not be ruled out. When passed through guinea-pigs, B. M. 5 became more virulent but, after successive passages, did not agglutimate so well; in fact, I found that it was not advisable to pass this strain through a guinea-pig, as most writers advocate doing to keep the strain agglutinable. Other strains, however, have not proved so constant as this one.

Next in importance to the native agglutinability and constancy of the strain, is the medium on which it is grown. The medium which has given the most satisfactory results is potato-glycerin-veal agar that is 2.5 per cent. acid to phenolphthalein. It is prepared as follows:

Veal infusion* (unadjusted)	1000 cc.
Agar	30 grams
NaCl	5 grams
Pepton (Fairchild)	10 grams

Put in autoclave or Arnold to melt agar.

Titrate at room temperature and adjust the *natural* acidity, if necessary, to Ph. 5.8 to 6.0 (2.5 acid phenolphthalein) by adding normal sodium hydroxid. *Do not add acid* under any circumstances.

Clear with egg and filter. Titrate again and if necessary, adjust to Ph. 5.8 to 6.0 (2.5 acid). Add glycerin (C. P.) 5 per cent. and potato juice 5 per cent. Sterilize in autoclave for one-half hour at 15 pounds pressure.

The potato juice used in this medium is prepared by adding one pound of unpealed potatoes, sliced thin, to 1 liter of distilled water. Autoclave one-half hour at 15 pounds, strain through cheese-cloth and a thin layer of cotton. Bottle for stock and sterilize in autoclave one-half hour at 15 pounds. This stock must be filtered each time before adding to medium.

That too much stress cannot be laid on the careful preparation of the medium is shown by the following experience. At one time in the beginning of my work, the suspension of B. M. 5 grown on a freshly prepared medium did not give the proper reactions with the control sera. Keeping in mind that the strain was old and in need of rejuvenation, I passed it through a guinea-pig and recovered the organism from the heart's blood. Meanwhile, a second fresh medium was made upon which the newly recovered organism was planted. This suspension reacted very successfully and I should have believed that the guinea-pig passage was responsible for the success had I not planted the old organism on the second medium also. The suspension of this growth worked as well as the one just isolated from the guinea-pig. Therefore, the old culture is still used.

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* Veal infusion: Chopped lean beef 10 pounds, water 10 liters. Soak over night. Heat for one hour at 45° C. then boil for one-half hour. Strain through cheesecloth. This infusion may be sterilized and stored for stock or used at once.

Careful attention should be given to all the glassware used in connection with cultures as well as the tests. It should always be neutralized. Bichlorid of mercury should be avoided as a disinfectant for the pipettes, bottles, tubes, etc.

The stock culture of *B. mallei* should be transplanted every ten to twelve days and incubated at 37.°C. for two or three days. It should then be kept in the ice-box at 10°C.

The suspension for the tests is prepared in the following manner: Forty-eight hour cultures are used to inoculate agar slants of the potato-glycerin-veal agar described above. A good growth should be insured by inoculating the surface generously. After forty-eight hours incubation at 37°C. the growth is washed off with 0.85 per cent. salt solution and killed by heating at 60°C. for two hours. No carbolic acid is added to this stock suspension which is of considerable density. It can be kept in the ice-box for two months or more in 100 c.c. bottles, corked and capped, if handled with aseptic precautions. The tests are carried out with a fresh dilution of the stock suspension made by adding 0.85 per cent. saline solution.

A sample dilution, which has been tested with known negative and positive sera, should always be kept in the ice-box and the fresh dilutions of the suspension compared with it on printed matter. The fresh suspension (dilution) is not filtered as the filtering seems to hold back something essential to the reaction; moreover, this is not necessary as it can be shaken up to a perfectly homogeneous fluid.

The tests are carried out in the following manner: A primary dilution of the serum 1:40 is made. Each tube receives varying quantities of this solution to which 3 c.c. of the bacterial suspension (prepared as directed above) is added to make a final serum dilution of 1:500, 1:800, 1:1000, 1:1200, 1:1600, 1:2000. A known positive and a known negative serum are always used as controls with each test; also a control tube of the bacterial suspension without serum. The tubes, in copper racks are placed in a water-bath (37° to 42°C.) for two hours.

With this technic a reaction up to 1000 or more may be obtained in ten to twenty minutes; a positive reaction always appears in two hours. The tests may be kept over night in the ice-box and read again in the morning. The reaction, if complete, is designated by two plus signs; incomplete, by one plus; slight, by plus minus, negative by a dash. The reaction is so clear cut there is no difficulty in interpreting it. One can watch the bacilli clump through the tube, then fall to the bottom in a white granular mass, leaving the supernatant fluid crystal clear in a complete reaction. There is no necessity to look for "buttons," "veils" or "films." It is either an agglutination as we see it with other organisms or it is not considered a reaction.

In reading the tests, a reaction is considered as *positive* which has double plus through 1:1600; as *suspicious* with double plus in 1:1200; as *doubtful* with double plus in 1:1000. Any reactions below 1:1000 are considered as negative.

It is essential to titrate the dilute bacterial suspension often with known positive and negative sera to be sure it is working. Tests should never be done with a suspension that has not been titrated the previous day.

The Department of Health of the City of New York has been employing the agglutination test for the diagnosis of glanders together with the complement fixation and ophthalmic mallein tests. During the last year, over 2000 sera were examined, which would have given us very rich material for arriving at definite conclusions as to the comparative value of the serodiagnostic and ophthalmic tests, checked by autopsy findings, had there been closer coöperation between the city and state veterinarians and the laboratory. Such coöperation should be based on a systematic method of recording in a central file full data in connection with each horse. For a thorough study of glanders in any region, each horse should be marked and registered under an identifying number, as are automobiles, only in this case the number should be a permanent one which should pass with the horse when it is sold. The traditional methods of horse dealers might make this a difficult matter, yet, for economic reasons a law to that effect rigidly enforced, would be thoroughly worth while.

On only 180 horses was it possible to obtain complete data which I have endeavored to analyze. These horses fall into three groups as shown in tables 1, 2, 3.

Street railway horses	111
Horses from an infected stable	14
Horses for slaughter	55
Total	180

The street railway horses and those from a badly infected stable (125 in all) are considered together as at autopsy they all proved to have glanders with the exception of two. These two were in the infected stable and they were killed with the others because of their marked exposure to the disease. One of these horses, number 562, table 2, had been given vaccine; thus the number of 125 is reduced to 123 on which the percentages are based.

The 55 horses for slaughter were among those that received careful tests to determine their fitness to be used for food. These horses came under the supervision of Dr. L. Price, veterinarian of the Department of Health; therefore the data are the most satisfactory of all.

Lately, we have been trying to confirm autopsy findings by pathological sections. This promises to be of great advantage. Many lesions which are suspicious of glanders do not prove so microscopically.

The bacteriological inoculation of suspicious material into guinea-pigs for the Strauss reaction does not prove of much aid, as, according to Miessner (3), it is successful in only 25 per cent. of the cases. My own experience is in accordance with this estimate. Only in acute cases (human) when *B. mallei* was isolated from the blood, or in horses with an acute infection of glanders was the Strauss reaction obtained in the guinea-pig after inoculation with emulsified material from the lesions. All other inoculation tests resulted negatively.

In considering the horses, in tables 1 and 2, the necessity of applying all three tests stands out. Of the 123 horses which proved at autopsy to have glanders, 122 were shown to be positive, suspicious or doubtful by one, two or three of the tests. Only one case (horse 123) was negative with all three tests—a failure of about 0.8 per cent.

The *agglutination* test showed 31 horses to be positive, 15 suspicious and 32 doubtful, making a total of 78 horses or 64.2 per cent. Thus it failed in 35.8 per cent. of the cases, probably because most of the horses in this series were chronic cases. Three horses[3] were picked out by this test alone—the complement fixation and eye mallein tests being negative. These may have been acute cases.

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[3] Table 1, horses, 76, 1218, 1504.

The *complement fixation* test showed 74 horses as positive, 4 suspicious and 17 doubtful, making a total of 93 horses or 75.6 per cent. This test failed wholly in 24.4 per cent. of the cases. Four horses[4] were picked out by this test alone.

On the other hand the *mallein* test (ophthalmic except for 9 horses in table 2 on which subcutaneous mallein was used) gave 39 horses as positive, 65 suspicious and 2 doubtful, making a total of 106 horses or 87.8 per cent. It failed in 12.2 per cent., but 15 horses[5] were picked out only by this test.

The grouping of suspicious and doubtful reactions with the positive ones is based on the interpretation that any serodiagnostic reaction not negative, should place an animal in the suspicious category to be subjected to close observation and frequent retests. That such a method is of great value is fully shown in tables 1 and 2.

Further Analysis.

Positive by all three tests	9 horses[*]
Positive by agglutination and negative complement fixation	6 horses[†]
Positive by complement fixation and negative by agglutination	23 horses
Positive by mallein and negative by complement fixation and agglutination	15 horses[‡]

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[*] Table 1, horses, 418, 506, 1776; Table 2, 645, 683, 738, 746, 750, 757.

[†] Table 1, horses, 163, 181, 605, 1218, 1360, 1504.

[‡] Table 1, horses, 19, 449, 462, 494, 612, 621, 1143, 1146, 1310, 1374, 1390; Table 2, horses, 730, 737, 751, 763.

Of the 55 horses tested before slaughter for food purposes (table 3), 4 horses[6] proved to have glanders. The complement fixation test failed to detect this fact in all 4 cases while the agglutination test failed in 3 of them and the ophthalmic mallein in only 1 (horse 2001) which the agglutination reaction picked out. The agglutination test gave 3 false "suspicious" reactions,[7] the complement fixation none, and the eye mallein 9.

In a set of horses such as are listed in tables 1 and 2, one may expect a large number of cases to be chronic and the agglutination tests to be positive in the least number of instances. On the other hand in a large number of sera examined during a whole year where different stables are tested there may be a great many cases of beginning infection or cases in the acute stages of the disease.

There were tested 1890 sera from various sources and among these the agglutination test gave positive and suspicious reactions in a larger proportion than the complement fixation. Unfortunately the eye mallein and final outcome of the cases could not be obtained.

Thus, with the *complement fixation,* about 14.5 per cent. of the cases were either positive or suspicious while the *agglutination* registered 28.2 per cent. The doubtful reactions occurring with two tests were almost the same in number (complement fixation 300 and agglutination 291).

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[4] Table 1, horses, 570, 1281, 1389, 2047.

[5] Table 1, horses, 19, 449, 462, 494, 612, 621, 1143, 1146, 1310, 1374 and 1390; Table 2, horses, 730, 737, 751, 763.

[6] Table 3, horses, 2001, 2006, 3044, 3064.

[7] Table 3, horses, 3027, 3040, 3053.

With complement fixation test

Positive	254
Suspicious	21
Doubtful	300
Negative	1315

With agglutination test

Positive	262
Suspicious	171
Doubtful	291
Negative	1166

With both tests

Positive	138
Suspicious	5
Doubtful	66
Negative	800

With either one of the two tests

Positive	378
Suspicious	187
Doubtful	525
Negative	800

These 1890 sera did not represent 1890 horses for about 15 per cent. were retests. If it had not been for these retests, a greater percentage of agglutination reactions would have been obtained for, after a lapse of time, the agglutinins disappeared in the horses retested, while the complement fixation antibodies remained, or appeared for the first time.

Another factor which influences the sero-diagnostic tests is the subcutaneous injection of mallein. It is generally held that six to eight weeks should elapse before the blood is taken for tests after mallein has been injected subcutaneously. Recently, in a large number of horses that had been given subcutaneous mallein, many showed four plus complement fixation and positive agglutination reactions, when retested about six weeks later. At the time of the first test these same horses had shown either doubtful or negative results. These horses are being kept under observation and time will show whether the high titers in both tests were due to the subcutaneous injection of mallein or to a fresh infection.

Summary.

1. A method has been devised whereby a prompt clear cut macroscopic agglutination for the diagnosis of glanders can be obtained in two hours.
2. Fifteen strains of *B. mallei* were tested and one obtained which is constant in its agglutinability with positive sera.
3. The best culture medium is glycerin-potato-agar Ph. 5.8 to 6.0 (2.5 acid to phenolphthalein) carefully prepared (see text).
4. Great care must be used in the preparation of the stock suspension of *B. mallei* (see text). For the tests, *fresh dilutions* in 0.85 per cent. salt solution are made from the stock suspension. *No carbolic acid is added* to the stock or to the dilutions.

5. The agglutination test is valuable in the routine examination of horses. With this test nearly all early and acute cases can be detected and thus the spread of the disease can be prevented.

6. A negative reaction by a single agglutination test if not confirmed by the ophthalmic and the complement fixation tests does not prove the case negative; nor does a single negative result either by the complement fixation or the ophthalmic test. A positive reaction by any of the three methods is more significant than a negative one. All three tests must be performed in order to pronounce the horse negative.

7. In my experience with the present series of cases a reaction of less than 1 : 1000 by agglutination (if the other two tests are negative and if there are no clinical symptoms) is not indicative of glanders in the horse. If the reaction is above a 1 : 1000 the horse should be kept under observation; even if he does not show active glanders, the possibility of a carrier condition should be thought of. Isolation of such horses would be a very safe measure for preventing the infection among the other horses.

8. The bacteriological method of inoculating the guinea-pig with glanders material for the "Strauss" reaction is successful in only 25 per cent. of cases (active lesions).

9. Autopsy findings, if possible, should be confirmed by pathological sections. A great many cases are pronounced glanders on autopsy, which do not show typical lesions under the microscope.

10. All stables should be under the supervision of the City Health Department and glanders in horses should be put in the same category as typhoid, meningitis, etc., in man, if glanders in New York City is to be eradicated. Sera that come to the Health Department for diagnosis should be accompanied by a complete history of each horse. Follow up work should be possible.

11. Records of the three tests and autopsy findings should be accessible and some mark of identification for every horse should be devised by which all records of a horse can be compared. Only in some such way can a really scientific study of the disease be made and the right conclusions drawn.

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(5) Miessner: Über die Infektiosität von Organteilen rötziger Pferde und die Komplementbindungsreaktion beim Meerschweinchem, etc. Centralblatt für Bact, Orig. 1 Abt., 1912, 64: 121.

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TABLE 1.

STREET RAILWAY HORSES.

Horse No.	Complement Fixation.	Agglutination.	Ophthalmic Mallein.	Remarks and Autopsy.
36	Pos.	Doubt.	Susp.	Glanders; lesions in lung
155	Pos.	Neg.	Susp.	Glanders; slight lesions in lungs and pleura
164	Pos.	Neg.	Pos.	Glanders; slight lesions in lungs
285	Pos.	Doubt.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
392	Pos.	Susp.	Susp.	Glanders; slight lesions in lungs and pleura
540	Pos.	Pos.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
626	Pos.	Susp.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
649	Pos.	Pos.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
660	Pos.	Pos.	Susp.	Glanders; lesions in lung
791	Pos.	Neg.	Susp.	Glanders; slight lesions in lung
855	Pos.	Pos.	Susp.	Glanders; lesions in lung and pleura
969	Pos.	Neg.	Susp.	Glanders; lesions in pulmonary node, lungs and pleura
981	Pos.	Pos.	Susp.	Glanders; lesions in lungs
1034	Pos.	Susp.	Susp.	Glanders; lesions in lungs
1120	Pos.	Neg.	Susp.	Glanders; slight lesions in lungs
1186	Pos.	Doubt.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
1204	Pos.	Susp.	Susp.	Glanders; lesions in lungs, pulmonary node and liver
1232	Pos.	Doubt.	Susp.	Glanders; slight lesions in lungs
1368	Pos.	Neg.	Susp.	Glanders; lesions in lungs
1370	Pos.	Pos.	Susp.	Glanders; slight lesions in lungs and pleura
1571	Pos.	Neg.	Susp.	Glanders; slight lesions in lung, liver and pulmonary node
19	Neg.	Neg.	Pos.	Destroyed; glanders lesions found
42	Pos.	Neg.	Pos.	
63	Doubt.	Neg.	Susp.	
76	Neg.	Doubt.	Neg.	
123	Neg.	Neg.	Neg.	
152	Pos.	Susp.	Neg.	
163	Neg.	Pos.	Susp.	
166	Pos.	Neg.	?	
180	Doubt.	Susp.	Pos.	
181	Neg.	Pos.	Susp.	
192	Pos.	Susp.	Pos.	
275	Pos.	Susp.	Neg.	
296	Pos.	Susp.	Susp.	
341	Pos.	Pos.	Susp.	
351	Neg.	Doubt.	Pos.	
418	Pos.	Pos.	Pos.	
443	Doubt.	Doubt.	Susp.	
449	Neg.	Neg.	Pos.	
462	Neg.	Neg.	Susp.	
471	Pos.	Doubt.	Susp.	
484	Doubt.	Susp.	Susp.	
493	Pos.	Doubt.	Pos.	
494	Neg.	Neg.	Susp.	
506	Pos.	Pos.	Pos.	
541	Doubt.	Doubt.	Pos.	
570	Doubt.	Neg.	Neg.	
579	Neg.	Doubt.	Susp.	
583	Pos.	Doubt.	Susp.	
586	Doubt.	Doubt.	Pos.	
605	Neg.	Pos.	Susp.	
612	Neg.	Neg.	Susp.	
614	Pos.	Susp.	Pos.	
621	Neg.	Neg.	Susp.	

TABLE 1—Continued.

Horse No.	Complement Fixation.	Agglutination.	Ophthalmic Mallein.	Remarks and Autopsy.
654	Pos.	Doubt.	Neg.	Destroyed; glanders lesions found
670	Susp.	Doubt.	Susp.	
679	Pos.	Doubt.	Susp.	
699	Pos.	Pos.	Susp.	
759	Pos.	Doubt.	Susp.	
818	Doubt.	Pos.	Susp.	
820	Doubt.	Doubt.	Susp.	
836	Pos.	Neg.	Susp.	
850	Pos.	Neg.	Susp.	
867	Pos.	Doubt.	Susp.	
871	Neg.	Doubt.	Pos.	
878	Doubt.	Doubt.	Susp.	
913	Pos.	Susp.	Neg.	
922	Pos.	Neg.	Pos.	
925	Pos.	Doubt.	Pos.	
962	Pos.	Neg.	Pos.	
963	Pos.	Doubt.	Neg.	
973	Pos.	Doubt.	Neg.	
1001	Pos.	Neg.	Pos.	
1018	Neg.	Doubt.	Susp.	
1055	Pos.	Neg.	Pos.	
1066	Pos.	Neg.	Susp.	
1072	Pos.	Doubt.	Neg.	
1074	Pos.	Neg.	Susp.	
1082	Pos.	Doubt.	Pos.	
1088	Pos.	Pos.	Neg.	
1121	Susp.	Pos.	Pos.	
1139	Pos.	Pos.	Susp.	
1143	Neg.	Neg.	Susp.	
1146	Neg.	Neg.	?	
1161	Doubt.	Susp.	Susp.	
1163	Pos.	Pos.	Susp.	
1164	Pos.	Neg.	Susp.	
1178	Pos.	Pos.	Susp.	
1199	Pos.	Doubt.	Pos.	
1207	Pos.	Susp.	Susp.	
1215	Susp.	Neg.	Susp.	
1218	Neg.	Pos.	Neg.	
1219	Doubt.	Doubt.	Susp.	
1281	Pos.	Neg.	Neg.	
1305	Neg.	Doubt.	Susp.	
1310	Neg.	Neg.	Susp.	
1360	Neg.	Pos.	Susp.	
1374	Neg.	Neg.	Susp.	
1389	Pos.	Neg.	Neg.	
1390	Neg.	Neg.	Susp.	
1393	Doubt.	Doubt.	Susp.	
1395	Doubt.	Susp.	Pos.	
1423	Pos.	Neg.	Susp.	
1504	Neg.	Pos.	Neg.	
1776	Pos.	Pos.	Pos.	
1784	Doubt.	Neg.	Pos.	
1880	Pos.	Neg.	Pos.	
1906	Pos.	Pos.	Neg.	
2031	Pos.	Neg.	Pos.	
2047	Doubt.	Neg.	Neg.	
2048	Pos.	Pos.	Susp.	

Complement Fixation: Positive, = 4 + and 3 +; suspicious, = 2 +; doubtful, = 1 +; negative, = ± and —.

Agglutination Positive, = 2 + in 1:1600 or over; suspicious, = 2 + in 1:1200; doubtful, = 2 + in 1:1000; negative, = any reactions below 1:1000.

TABLE 2.

HORSES FROM INFECTED STABLE.

Horse No.	Complement Fixation.	Agglutination.	Ophthalmic Mallein.	Subcutaneous Mallein.	Remarks and Autopsy.
645	Pos.	Pos.	....	Pos.	Glanders; lesion in pulmonary node
683	Pos.	Pos.	Pos.	....	Glanders; lesion in pulmonary node
730	Neg.	Neg.	....	Pos.	Glanders; lesion in pulmonary node
737	Neg.	Neg.	....	Pos.	Glanders; lesion in pulmonary node
738	Pos.	Pos.	Pos.	....	Glanders; lesions in pulmonary node
745	Susp.	Neg.	....	Pos.	Glanders; lesion in pulmonary node
746	Pos.	Pos.	....	Pos.	Glanders; lesions in pulmonary node
747	Doubt.	Doubt.	....	Pos.	Glanders; pulmonary and nasal lesions
750	Pos.	Pos.	Pos.	....	Glanders; lesion in pulmonary node
751	Neg.	Neg.	....	Pos.	Glanders; nasal lesion only
757	Pos.	Pos.	....	Pos.	Glanders; lesions in pulmonary gland
763	Neg.	Neg.	....	Pos.	Glanders; pulmonary and nasal lesions
762	Susp.	Neg.	Neg.	Neg.	No glanders lesions; caseous material in one bronchus
562	Neg.	Pos.	....	Pos.	Had been given vaccine. No glanders lesions found

TABLE 3.

HORSES FOR SLAUGHTER.

Horse No.	Complement Fixation.	Agglutination.	Ophthalmic Mallein.	Intrapalpebral Mallein.	Remarks and Autopsy.
2001	Neg.	Pos.	Neg.	....	Lesions in lung. Pathological sections showed *glanders* nodules
2002	Neg.	Neg.	Neg.	....	Cyst in liver
2003	Neg.	Doubt.	Neg.	.....	Healed pneumonic lesions
2004	Neg.	Doubt.	Neg.	....	Emphysema; multiple cysts in liver
2005	Neg.	Neg.	Neg.	....	No autopsy
2006	Neg.	Neg.	Pos.	Pos.	*Glanders:* encapsulated lesions with gelatinous infiltrated pneumonic areas
2007*	A.C.	Doubt.	Neg.	....	No glanders lesions
3008	Neg.	Doubt.	Neg.	....	Few calcified parasitic lesions in liver
3009	Neg.	Neg.	Neg.	....	Extensive parasitic lesions in liver
3010	Neg.	Neg.	Neg.	....	Lungs—alveolar emphysema
3011	Neg.	Neg.	Neg.	....	No glanders lesions
3012	Neg.	Neg.	Neg.	....	No glanders lesions
3013	Neg.	Neg.	Susp.	Neg.	No autopsy. Submaxillary gland tubercular?
3014	Neg.	Neg.	Neg.	....	No glanders lesions
3015	Neg.	Neg.	Neg.	....	Multiple cysts in liver; adhesions lung
3016*	A. C.	Neg.	Neg.	....	Encapsulated area of parasites
3017	Neg.	Neg.	Neg.	....	Parasitic lesions in liver
3018	Neg.	Neg.	Susp.	....	Parasitic lesions in lungs
3019	Neg.	Doubt.	Neg.	....	No glanders lesions
3020	Neg.	Neg.	Neg.	....	Calcified brown area in lung; parasitic liver
3021	Neg.	Neg.	Neg.	....	Liver parasitic; organized haematoma in coecum

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* Mules.

TABLE 3—Continued.

Horse No.	Complement Fixation.	Agglutination.	Ophthalmic Mallein.	Intrapalpebral Mallein.	Remarks and Autopsy.
3022	Neg.	Neg.	Neg.	....	Fibrous nodule in lung
3023	Neg.	Neg.	Neg.	....	Parasitic liver
3024	Neg.	Neg.	Neg.	....	No glanders lesions
3025	Neg.	Neg.	Neg.	....	Parasitic liver
3026	Neg.	Neg.	Susp.	....	Chronic pleuritis
3027	Neg.	Susp.	Susp.	....	Cirrhosis of liver; endocarditis
3028	Neg.	Neg.	Neg.	....	No lesions of glanders
3029	Neg.	Doubt.	Neg.	....	Liver cystic. No glanders lesions
3030	Neg.	Neg.	Susp.	....	Suspicious lesions in lung. Pathological sections showed no glanders lesions microscopically
3031	Neg.	Neg.	Neg.	....	No lesions of glanders
3032	Neg.	Neg.	Neg.	....	No glanders lesions
3033	Neg.	Neg.	Neg.	....	No glanders lesions
3034	Neg.	Doubt.	Neg.	....	Parasitic lesions in lungs and liver
3035	Neg.	Neg.	Neg.	....	No glanders lesion
3036	Neg.	Neg.	Neg.	....	No glanders lesions
3037	A. C.†	Neg.†	Susp.	....	Purulent broncho-pneumonia
3038	Neg.	Neg.	Susp.	....	Lymphatic nodes—calcified lesions; liver—parasitic lesions. Microscopic sections negative
3039	Neg.	Neg.	Neg.	....	Cirrhotic liver
3040	Neg.	Susp.	Neg.	....	Liver—calcified lesions
3041	Neg.	Neg.	Neg.	....	Liver—parasitic lesions
3042	Neg.	Neg.	Neg.	....	Liver—parasitic lesions
3043	Neg.	Neg,	Neg.	....	No glanders lesions
3044	Neg.	Pos.	Pos.	....	Typical *glanders* lesions
3045	Neg.	Neg.	Neg.	....	Pigmented deposits in lungs
3046	Neg.	Neg.	Neg.	....	Parasites in liver
3047	Neg.	Neg.	Neg.	....	Parasites in liver
3048	Doubt.	Doubt.	Susp.	....	Lungs, spleen, liver and nodes—suspicious lesions. Pathological sections show no glanders lesions
3049	Neg.	Neg.	Neg.	....	Parasitic nodules in liver
3050	Neg.	Neg.	Neg.	....	Parasitic nodules in liver
3051	Neg.	Pos.	Neg.	....	Suspicious lesions in lungs. Pathological sections show no glanders lesions
3052	Neg.	Doubt.	Neg.	....	Slight adhesions of diaphragm
3053	Doubt.	Susp.	Susp.	....	Slightly suspicious lesions in lungs. Gelatinous infiltration of abdominal wall. Pathological sections show no glanders lesions
3055	Neg.	Neg.	Neg.	....	Parasitic liver and lung
3064	Neg.	Neg.	Pos.	....	*Glanders* lesions in lung

† Two tests.

THE STANDARDIZATION OF GUINEA-PIG SERUM FOR COMPLEMENT FIXATION TESTS, ESPECIALLY IN REGARD TO THE TITRATION OF THERAPEUTIC IMMUNE MENINGOCOCCUS HORSE SERUM.*

M. A. Wilson.

In the work of standardizing therapeutic immune meningococcus horse sera by complement fixation so many difficulties have been encountered in the attempt to determine the accurate anti-body content, that the interest of our department has been largely centered upon this subject for the past two years.

The tests upon which the standardization of a new serum is based, consist of titrations of that serum in comparison with the titrations of known sera. In order that the comparison may be true, it is necessary that the reagents used in making the tests shall be as nearly constant in their reactions as possible, leaving as the only variable the unknown serum to be tested. This would be a simple matter, were it not for the fact that the complement is also a variable factor, and attempts to render it constant are not always successful. It is true then in this, as in all other complement fixation work, that the value of the tests depends upon the accurate measurement of the dose of complement to be used.

Formerly this complement dose was measured in regard to its hemolytic strength, an exact hemolytic unit being used with two hemolytic units of the amboceptor. If the fixation and hemolytic units of a given complement were always proportionate, this method for the determining the complement dose would be a good one, as a clear-cut hemolytic reaction is quickly and easily obtained if the complement is in good condition. Unfortunately for the convenience of the technician, the hemolytic and fixation strengths of a guinea-pig serum are in many instances diverse, and to measure the dose of the complement by its hemolytic unit will not give the accurate titre of the unknown serum. This fact was demonstrated so many times that experiments were undertaken to obtain a method which would give uniform results.

The fundamental idea in our experiments has been to determine the fixation strength of the complement to be used, and to adjust the hemolytic system to meet the requirements of that fixation strength.

After repeated experiments in comparison with the old method, we have found the following technique to give uniform results. This method is now in use for all our standardization tests:

First of all it is essential that the guinea-pigs be healthy, large and firm, not fat. The pigs are kept for an hour or two without food before killing. They are killed by bleeding them to death from the carotid arteries, the blood being collected in sterile, neutral Petri dishes. The serum is removed from the clot after it has stood in the ice-box overnight.

I might say here that we have tried a number of experiments to determine whether it is best to leave the serum on the clot for several hours, or to remove it as soon as possible. We have found that the complement is more efficient for hemolysis and fixation when left on the clot over night than when left for two or three hours only. In this we have verified the reports of earlier workers. The serum from each pig is collected separately and tested separately before pooling.

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The following are the tests made:

1. Test for natural antisheep amboceptor.
2. Test for hemolysis with a known hemolytic amboceptor.
3. Test for anticomplementary reaction with meningococcus antigen.
4. Test for anticomplementary reaction with meningococcus serum.
5. Test for anticomplementary reaction with heterologous serum.
6. Test for hemolytic reaction with meningococcus antigen, less hemolytic amboceptor.
7. Test for hemolytic reaction with meningococcus serum, less hemolytic amboceptor.
8. Test for fixation with the combination of meningococcus antigen with meningococcus serum.
9. Test for lysis of cells by antigen alone.
10. Test for lysis of cells by serum alone.

The importance of making these preliminary tests before pooling the complement from several pigs has been shown in our daily tests of the complement to be used both for meningococcus and gonococcus fixation. Many times in a lot of complement from ten pigs to be pooled, several will not be fixed by the combination of meningococcus antigen and serum, and the same is true with the gonococcus combinations. Out of 500 guinea pigs killed only 75 per cent. were efficient for meningococcus fixation. Therefore, if accurate results are to be obtained with these antigens and sera, the preliminary tests must be made. The most serious objection to this work is the fact that it takes so much time, and also an extra amount of complement; but, when one considers the amount of material and time wasted when tests are made with complement which has not been accurately tested, the additional expenditure would seem to be justified.

After the results of the preliminary tests are obtained, the sera which have been found to have adequate fixation and hemolytic strength for meningococcus fixation are pooled, and the pooled complement is tested to determine the exact complement dose to be used. This is accomplished by titrating the standard dilution of a known meningococcus serum with two standard units of meningococcus antigen and with varying dilutions of the pooled complement. For the standard dose of complement to be used in testing the unknown sera, we use 0.1 c.c. of that dilution of the complement which gives the standard titre of the known serum in the required fixation time and which gives complete hemolysis of all controls within thirty minutes.

The following table shows the variation in titre of one bleeding of meningococcus horse serum when tested with complement standardized by the old and new methods. The standard titre of this bleeding was .0004 c.c.

Titration of unknown sera: Having found the most favorable dose of complement, the unknown sera are titrated with this in the presence of two standard units of the meningococcus antigen. The tests are placed in the water bath for thirty minutes for the fixation of the complement. At the end of the fixation period the indicator, consisting of .1 c.c. of a 5 per cent. suspension of sheep cells sensitized with two units of hemolysin, is added, and the tests are replaced in the water bath for 1 hour. The re-

TABLE 1.

PRELIMINARY TESTS.

Test No.	Mening. Serum.	Mening. Antigen.	Hetero-logous Serum.	Comple-ment 1:10 Dil.	Sheep Cells 5% susp.	Sensi-tized Cells.	Saline.
1	None	None	None	.2 c.c.	.1 c.c.	None	To bring volume to .5 c.c.
2	None	None	None	.1 c.c. .05 c.c.	None	.1 c.c. sens. with 2 units amb.	To bring volume to .5 c.c.
3	None	.2 c.c. Standard Dilution	None	.1 c.c.	None	1. c.c. sens. with 2 units amb.	To bring volume to .5 c.c.
4	.04 undiluted	None	None	.1 c.c.	None	.1 c.c. sens. with 2 units amb.	To bring volume to .5 c.c.
5	None	None	.04 undiluted	.1 c.c.	None	.1 c.c. sens. with 2 units amb.	To bring volume to .5 c.c.
6	None	.2 c.c. Standard Dilution	None	.1 c.c.	.1 c.c.	None	To bring volume to .5 c.c.
7	.04 undiluted	None	None	.1 c.c.	.1 c.c.	None	To bring volume to .5 c.c.
8	2 Standard units	.1 c.c. Standard Dilution	None	.1 c.c.	None	.1 c.c. sens. with 2 units amb.	To bring volume to .5 c.c.
9	None	.2 c.c.	None	None	.1 c.c.	None	To bring volume to .5 c.c.
10	.04 undiluted	None	None	None	.1 c.c.	None	To bring volume to .5 c.c.

TABLE II.

OLD AND NEW METHODS COMPARED.

	Number of Guinea-pigs Killed.	Number Efficient for Meningococcus Fixation.	Hemolytic Unit of Pooled Complement.	Fixation Strength of Pooled Complement.	Titre of Meningococcus Horse Serum.	Remarks.
OLD METHOD	10	No test for fixability	.006 c.c.	No test	.004 c.c.	One hemolytic unit gave standard titre of horse serum.
	6	No test for fixability	.005 c.c.	No test	No complete fixation	One hemolytic unit did not give standard titre.
	10	No test for fixability	.007 c.c.	No test	.001 c.c	One hemolytic unit did not give standard titre.
	6	No test for fixability	.008 c.c.	No test	.0008	One hemolytic unit did not give standard titre.
NEW METHOD	10	4	.005 c.c.	.007 c.c.	.0004 c.c.	One fixation unit gave standard titre.
	6	All	.008 c.c.	.008 c.c.	.0004 c.c.	One fixation unit gave standard titre.
	6	5	.004 c.c.	.005 c.c.	.0004 c.c.	One fixation unit gave standard titre.
	6	3	.004 c.c.	.005 c.c.	.0004 c.c.	One fixation unit gave standard titre.

actions may be read at the end of the hour, or the tests may be placed in the ice-box over night and the reactions read from the supernatant fluid in the morning. If the latter method is used, it is essential to have a color control of each serum, otherwise the reactions may be inaccurately read. The reason for this is that some of the sera have enough color, ranging from a pale lemon to a deep orange, to give a tinge of yellow to the supernatant fluid, which if read in comparison with a serum having no color would give an inaccurate titre. I am in favor of reading at the end of the hour when titrating therapeutic immune sera, according to the method employed for diagnostic tests.

Application of this method to a diagnostic test:—The standardization of the complement by this method has not been applied in tests of patients' sera for diagnostic purposes, except as a matter of experiment, and not enough work has been done upon that phase of the test to justify a statement as to its value. However, I can say without hesitation that the preliminary tests of the pigs' sera before pooling show a marked difference in their reactions with the gonococcus and tuberculosis antigens.

In the work reported here we have included no reactions in which all the controls were not completely hemolyzed, the hemolytic reaction reading being compared to a control of cells hemolyizized by the addition of water. The fixation reaction readings have been controlled by a suspension of cells in saline, thus ruling out, as far as possible the question of personal equation in reading.

Points of difference between this and the ordinary laboratory method:—The only point of difference is that we have based our measurement of the complement dose upon its fixability and not upon its hemolytic strength; and that before pooling the sera from a number of pigs we have tested each one for its fixability with the antigen and serum combination. The hemolytic strength is taken into account, and also the anticomplementary reaction with various reagents, as has been shown in our description of preliminary tests of the sera before pooling.

Advantages of this Method:—By making the preliminary tests before pooling and by finding the exact fixation strength of the pooled complement before proceeding to make the tests of the unknown sera, much time and material are saved, and an accurate titre of the unknown serum is obtained.

Conclusions.

I. All guinea pig complements are not fixed by the combination of meningococcus antigen and meningococcus antibody.

II. Before being pooled for meningitis tests, the serum from each guinea-pig should be tested for fixability with meningococcus antigen and antibody.

A CONTRIBUTION TO THE STUDY OF THE COMPLEMENT FIXATION REACTION IN TUBERCULOSIS.*

M. A. Wilson.

Preliminary Report—

Part I. On the standardization of complement.
Part II. Observation of the von Wedel Reaction.

Part I.

In our study of tuberculosis complement fixation, we have found a point of technique which has increased the efficiency of the test. This has to do with the standardization of the guinea-pig-serum to determine the value of the complement. In this preliminary report we shall describe the method for standardizing the complement, the preparation of our tuberculosis antigen, and the diagnostic test, with such results as we have obtained thus far in our study.

Technique.

All reagents are used in one-tenth the classic Wasserman volumes. Fixation period, one hour at 37° C.

Serum—The patient's serum is inactivated for thirty minutes at 56° C. .02 c.c. and .01 c.c. of the undiluted serum are used in the test, and .04 c.c. for control of anticomplementary reaction.

Antigen—Two antigens have been used. One was made from 12 stock cultures of human tubercle bacilli, the other from human strain 305 (used for tuberculin production). These antigens are suspensions in .9 per cent. saline of dried bacilli, from which all constituents soluble in alcohol and ether have been removed. The antigens were grown in glycerin-broth. As it happened the polyvalent antigen cultures were grown for three weeks, and the monovalent for three months, the only difference in the antigen produced being that the longer growth gives a more potent product, permitting higher dilution. The broth cultures were killed by heating in the Arnold sterilizer for one hour. The cultures were then filtered through filter paper. The filtrate was discarded and the residue placed in absolute alcohol, in the proportion of one volume residue to 10 volumes alcohol. This mixture was shaken thoroughly by hand, and placed in the ice-box for two weeks. It was then filtered through paper; the filtrate discarded and the residue washed in absolute alcohol. After this it was centrifugalized and washed in ether, centrifugalized again and the ether discarded. The centrifuge tube containing the residue was plugged and placed in the dark at room temperature over night. By this simple procedure the residue was dried within 24 hours. The dried powder was emulsified in a large mortar was .9 per cent. saline in proportion of 1 gram of powder in 200 c.c. of saline. This gave a concentrated emulsion convenient for storing as a stock antigen. The emulsion was heated for one hour at 80° C. The antigen was now ready for use, and was standardized to be used in such a dilution that .1 c.c. contained two standard fixation units and one fourth, or less of the anticomplementary dose. The unit was determined by titrating varying amounts of the antigen with .01 c.c. of a known positive tuberculosis serum, and two hemolytic units of a complement known to be potent for tuberculosis fixation.

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As stated above two antigens were used in our tuberculosis tests. The standard dilution of these two antigens is 1:50. This makes a final dilution of dried bacilli 1:10,000. These antigens are not anticomplementary. They have given uniform and constant fixation reactions. The tests reported will show that they are specific and stable. They were made ten months ago and are perfectly efficient today.

Complement—Guinea-pig's serum, twenty-four to forty-eight hours old; pooled from six to ten pigs. Before pooling, the serum from each pig is tested: 1, for hemolytic strength; 2, antisheep amboceptor; 3, anticomplementary reaction with a heterologous serum, and 4, fixability with the combination of tuberculosis antigen and tuberculosis serum. This last test we emphasize as an essential, if uniform results are to be obtained with different lots of complement. It has proven true beyond a doubt that although a guinea-pig serum may react perfectly in all other respects it may fail to be fixed by tuberculosis antigen and serum. Of the 129 pigs we have tested only 36 per cent. were efficient; 64 per cent. failed in fixability, although they were perfectly good in other respects. If in a pool of six or ten complements, there are several strongly fixable, the presence of the negative complements in the pool may not appreciably affect the test; on the other hand, if most of the complements are negative the fixability of the pool will not serve to give a true reaction with the patient's serum. A four plus may drop to a two or three plus, and a two or one plus may be missed.

Tables 1 and 2 show the variation in fixability of the guinea-pig serum.

TABLE I.

Pig No.	Complement Fixation Reaction.		
	Tuberculosis.	Meningococcus.	Gonococcus.
1	No fixation	Complete fixation	Complete fixation
2	No fixation	Complete fixation	Complete fixation
3	Complete fixation	Complete fixation	Complete fixation
4	No fixation	Complete fixation	Complete fixation
5	Weak fixation	Complete fixation	Complete fixation
6	No fixation	Complete fixation	No fixation
7	No fixation	Complete fixation	No fixation
8	Weak fixation	Complete fixation	Complete fixation
9	No fixation	Complete fixation	Complete fixation
10	Weak fixation	Complete fixation	No fixation
11	Complete fixation	Complete fixation	Complete fixation
12	Complete fixation	Complete fixation	Weak fixation

NOTE—All pigs bled Nov. 13, 1917.

TABLE II.

SHOWING NUMBER OF GUINEA PIG-SERA EFFICIENT FOR TUBERCULOSIS COMPLEMENT FIXATION.

Number of Pigs Bled.	Number Efficient for Complement Fixation.		
	Tuberculosis.	Meningococcus.	Gonococcus.
10	1	10	4
3	3	3	3
11	1	11	6
8	8	8	8
11	3	10	8
6	1	No test	1
6	3	6	1
10	3	10	8
5	0	4	4
11	4	9	6
4	1	4	4
10	5	10	8
8	3	8	6
10	5	9	8
6	1	5	6
10	4	10	7
129	46	117	88

The pooled serum is titrated with cells sensitized with 3 standard units of amboceptor. The reaction is read at the end of 15 minutes. Two hemolytic units of the pooled complement are used.

Indicator for the fixation reaction—.1 c.c. of a 5 per cent. suspension of sheep cells sensitized with three standard units of antisheep amboceptor.

Controls.

Antigen—for anticomplementary reaction.
for specificity.
for potency.
Serum—for anticomplementary reaction.
for specificity.
for natural antisheep amboceptor.
Complement—for stability (system control).
Sensitized Cell—for stability (reading control).

RESULTS.

TABLE III.

RESULTS OF 336 TESTS.

	Pulmonary Active 12 Cases.	Bone and Joint Diseases 48 Cases.	Tubercular Glands 14 Cases.	Poliomyelitis Spinal Fluids 50 Cases.	Cases Having No Symptoms of Tuberculosis 212 Cases.
Strongly positive.	67%	12%	35%	0	0
Weakly positive..	25%	10%	23%	0	0
Negative........	8%	78%	42%	100%	100%

CONCLUSIONS,

First—All guinea-pig serums are not efficient for tuberculosis complement fixation.

Second—The serum from each guinea-pig should be tested for fixability with tuberculosis antigen plus tuberculosis serum before pooling the complement for diagnostic tests.

PART II.

Observation of the von Wedel Reaction.

Dr. von Wedel, working independently in our laboratory, found that some serums from active tuberculosis cases gave a negative complement fixation reaction when the test was made on the first day after bleeding. When the same specimens of serum were tested a week later, having stood in the ice-box during the interval, they gave a positive test. This occurred repeatedly on later bleedings from the same patient. The complement used for all tests had been previously tried for fixability with tuberculosis antigen plus tuberculosis serum, therefore, the negative reaction in the first test was not due to an inefficient complement. Serums from non-tubercular cases gave no fixation at any period after bleeding, and this fact rules out the question of non-specificity of the later positive reaction following the early negative phase.

The controls for anticomplementary reaction in the patient's serum and in the antigen were all completely hemolyzed.

This early negative phase was not demonstrated in the serums from all tubercular cases; but the percentage was so large as to be significant.

Having personally observed the accuracy of Dr. von Wedel's technique, and the many repeated tests he made to discover the possibility of an error, I was convinced of the verity of the reaction and of the necessity for making the later test before the tuberculosis antibody content of any serum can be determined.

All tubercular serums to be tested throughout the remainder of my study will be given the early and later tests.

THE PREPARATION AND STANDARDIZATION OF BACTERIAL ANTIGENS.

Methods Employed at the Research Laboratory, Department of Health, for the Preparation of Bacterial Antigens.

M. A. Wilson, Assisted by L. Alper, R. Shivitz, M. V. Forbes, R. M. Nedley, R. Slobodkin, and F. Schwartz.

We have received many inquiries regarding the preparation of our bacterial antigens, therefore this preliminary report is given with the hope that it may be of assistance to others who are working upon this subject. For the past two years the writer has had charge of the preparation of the bacterial antigens used in our laboratories. During this time many methods have been tried out and those which have given the most satisfactory results are described here. In no instance, however, has the antigen been perfect in every detail and further work is being done in the attempt to obtain as specific a product as possible.

Meningococcus Antigen.

This antigen has been used by us for the standardization of therapeutic immune horse sera. It has given excellent results in that work. Not one of our meningococcus antigens has become anticomplementary. They are stable after standing in the ice-box at 4° to 10°C. for twelve months.

Method of Preparation: Medium for stock culture. *Glycerine-veal-agar (reaction about Ph 7.4 or 0.6 per cent. acid to phenolphthalein) 3 vols.; sterile normal horse serum, 1 vol.

Medium for antigen plates: Salt-free-veal-agar, reaction neutral to phenolphathalein.

Reagents: 50 per cent. alcohol, absolute alcohol, 9 per cent. saline.

Cultures: Several strains of meningococcus not recently isolated. Transplant antigen cultures from the stock tubes to potato tube slants of glycerine-veal-horse-serum agar and incubate for twenty-four hours at 37°C. From these large agar slants transfer the growth to the surface of salt-free veal-agar plates, the reaction of the medium being neutral to phenolphthalein. In order to insure abundant growth on the plates, it is well to inoculate each plate with the whole growth from two potato tube agar slants. We have had better results in making the transplants with a platinum wire than with a swab. Incubate these antigen plates for twenty-four hours at 37°C. Make smears from each plate, stain by Gram's method and examine for purity of growth. In order to prevent contamination of plates during incubation, place a fold of cheese-cloth wrung out of bichloride solution over the top of the pile of plates and another on the shelf under the plates. Since we adopted this method very few plates have had to be excluded on account of contamination. All pure plates are treated in the following manner:

With a sterile glass slide scrape the growth to the center of the plate and with a sterile platinum loop lift the mass and place it in a centrifuge tube containing 10 c.c. of 50 per cent. alcohol. Remove the growth from all the plates and put into this tube. This method is an improvement

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* Formulae of the various media mentioned will be found at end of article.

upon that of washing the growth from the surface of agar in bottles or tubes. It avoids the inclusion of any culture medium and lessens the labor of the subsequent washings, since we have now the material in only one small centrifuge tube to handle. Stir the mixture thoroughly with a sterile glass rod and centrifuge for five minutes at high speed. Pour off the supernatant fluid and add 10 c.c. of absolute alcohol. Stir the mixture again thoroughly with a sterile glass rod, place in the water bath at 37°C. for thirty minutes and stir frequently. Next centrifugalize the mixture for five minutes and pour off the supernatant fluid. Then add to the sediment about 10 c.c. more of absolute alcohol, centrifugalize for five minutes and discard the supernatant fluid. Continue this washing until the supernatant fluid is as clear as water. To the sediment add ether 10 c.c., stir with a sterile glass rod, leave standing at room temperature for thirty minutes, and stir frequently. Centrifugalize for five minutes and pour off supernatant fluid. Then add to the sediment about 3 c.c. of ether, stir and centrifugalize for two or three minutes and pour off supernatant fluid. Plug the centrifuge tube and place at room temperature in the dark for twenty-four hours, or in the incubator until dry. Some of these bacterial products are very hydroscopic and if the sediment when dried has the appearance of a black, gummy mass, it is not a matter of ill-omen, as the moisture absorbed from the air does not injure the antigen but evaporates during the process of trituration.

First weigh the dried product and then grind it with 0.9 per cent. saline, in a mortar. Add saline in sufficient amount to bring the proportion of powder and saline to approximately 10 milligrams of powder for each cubic centimeter of saline. This will give an even suspension, which can be diluted later in accordance with the standard fixation unit obtained by titration of the antigen with an homologous serum.

Next place the suspension in a sterile, neutral flask or bottle, and heat at 80°C. for one hour. This is an important step in the successful treatment of any bacterial antigen.

Bottle the antigen, preferably in 5 c.c. amounts and sterilize at 56°C. for one hour on each of three successive days. Seal the bottles with paraffin and cap with capping skin. The antigen is now ready for use and is stored in an ice-box at a temperature of 4° to 10°C. It is not injured by freezing.

The method for standardizing the antigen is given later in this paper.

Efficient meningococcus antigens have been made with cultures grown upon Dopter's liver medium, disodium-phosphate-agar, salt-free-veal-agar, and Huntoon's beef-heart-agar.

Gonococcus Antigen.

This method for the preparation of gonococcus antigen is a modification of the method given to us by Dr. Archibald McNeil, and is similar to that described for meningococcus antigen.

Strains: The ten Torrey strains, A, B, C, G, K, L, N, O, Q, S, are used for the gonococcus antigen.

Medium for stock culture: Disodium agar. Reaction Ph 7.6.

Medium for antigen culture: Disodium agar or, the cultures for stock and antigen can be made on glycerine-veal-agar with horse serum. We have obtained excellent antigens with the latter medium.

Growth: Inoculate slants of disodium agar with stock cultures and incubate for forty-eight hours. Transplant the growth to large slants of disodium agar in potato tubes and incubate for forty-eight hours, at 37°C.

The further preparation of the antigen is the same in every detail as described for the meningococcus antigen.

Gonococcus antigens made by the above method are used in our laboratories for the routine diagnostic tests. Several other laboratories in New York, Boston and Philadelphia have used these gonococcus antigens also. We have received very favorable reports with regard to their specificity. The titre of such antigens varies from 1:20 to 1:40 and they are not anticomplementary.

GLANDERS ANTIGEN.

Medium for stock cultures: Glycerine-potato-veal-agar. Reaction Ph 5.8—6 or 1.6 per cent. acid to phenolphthalen.

Medium for antigen Plates: Salt-free-veal-agar, reaction neutral to phenolphthalein.

The stock cultures are trasplanted at intervals of one week, and are kept in the incubator at 37°C.

To prepare antigen, make transplants from stock cultures to the surface of glycerine-potato-veal-agar slants in potato tubes and incubate for twenty-four hours; from these fresh transplants inoculate the surface of salt-free-veal-agar plates and incubate these plates for twenty-four hours at 37°C.

Scrape the growth from the antigen plates and place it in a centrifuge tube containing 15 c.c. of 0.9 per cent. saline solution. Heat in Arnold sterilizer at 100°C. for one and one-half hours. Centrifugalize at medium speed for twenty minutes, extract in absolute alcohol at 37°C. for thirty minutes, and stir constantly. Centrifugalize for five minutes. Wash quickly in absolute alcohol. Centrifugalize for five minutes. Extract in ether at room temperature for thirty minutes. Centrifugalize for five minutes. Pour off supernatant ether, plug the centrifuge tube, and place in the dark at room temperature over night. Finally emulsify the dried powder in saline by the method used for meningococcus antigen.

TYPHOID ANTIGEN.

Antigen cultures are grown on salt-free-agar, reaction neutral to phenolphthalein, for twenty-four hours. The antigen is then made as follows:

Scrape the growth from the surface of plates into a centrifuge tube containing 15 c.c. of 0.9 per cent. saline solution. Place this tube in Arnold sterilizer for 1 hour at 100°C. Centrifugalize for ten minutes. Extract in absolute alcohol for thirty minutes at 37°C., and stir constantly. Centrifugalize for ten minutes. Wash quickly in absolute alcohol, and extract in ether for thirty minutes. Centrifugalize for five minutes and dry over night at room temperature. Emulsify the dried powder by the method for meningococcus antigen.

PERTUSSIS ANTIGEN.

Medium for stock culture: Bordet-Gengou glycerine-potato-blood-agar.

Medium for antigen: Glycerine-veal-horse-serum-agar, reaction Ph. 7.2 to 7.4 or 0.5 per cent. acid to phenolphthalein.

The stock cultures are transplanted at intervals of seven days. To prepare the antigen make transplants from the stock cultures to the surface of glycerine-veal-horse-serum-agar slants and incubate for thirty-eight hours at 37°C. From these fresh cultures inoculate plates of glycerine-veal-horse-serum-agar and incubate for forty-eight hours. Proceed further according to the method for making typhoid antigen.

INFLUENZA ANTIGEN.

Medium for stock cultures and antigen: Coagulated horse blood agar.

The stock cultures are transplanted at intervals of seven days, and grown on coagulated horse blood agar. For the antigen make cultures on the same medium from fresh stock cultures and incubate for forty-eight hours. From these last cultures streak plates of the same medium with a large amount of growth. Incubate the plates at 37°C. for forty-eight hours, remove the growth and prepare the antigen by the same method used for the meningococcus antigen.

STREPTOCOCCUS ANTIGEN.

Medium for stock cultures: Blood streaked agar slants.

Medium for antigen: Salt-free-veal-agar, reaction neutral to phenolphthalein.

The stock cultures are grown on blood streaked veal agar slants and transplanted at intervals of seven days. To prepare antigen make transplants from the stock cultures upon salt-free-veal-agar and incubate twenty-four hours at 37°C. From these fresh cultures inoculate plates of salt-free-veal-agar and incubate at 37°C. for twenty-four hours. Scrape the growth into a centrifuge tube containing 15 c.c. of a 0.9 per cent. saline solution.

The method for Tuberculosis Antigen is given in a separate article.*

THE STANDARDIZATION OF BACTERIAL ANTIGENS.

In order that there may be as little variation as possible in the antigens, each new product is titrated in comparison with one of known titre, and before the titrations of the new product are made the complement is adjusted to meet the requirements of the known antigen.

PRELIMINARY TEST TO DETERMINE THE COMPLEMENT DOSE.

Method: Dilute complement, 1:5, 1:10, 1:12, 1:15, 1:20. Titrate the standard dilution of the known antigen with two standard units of a known homologous serum, and with .1 c.c. of a 1:10 dilution of complement. Fix in the water bath at 37°C. for the time required according to the antigen in question. (Gonococcus fixation, two hours; meningococcus, thirty minutes; tuberculosis, one hour; pertussis, one hour, etc).

Add blood cells which have been sensitized with three standard units of amboceptor. Replace in the water bath for one hour. Read the antigen unit of fixation at the end of one hour.

In order to obtain satisfactory results with the complement pooled from the bleeding of several pigs, it is necessary before pooling, to test the serum of each pig, for: (1) hemolysis, (2) fixation with the antigen and serum combination to be used, (3) anticomplementary reaction with the antigen and serum and (4) hemolytic reaction with the antigen and serum.

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* See page 482.

Titration of the new antigen.—Dilute the new antigen 1:5, 1:10, 1:20, or such other dilutions as previous work would suggest as desirable.

Titrate each dilution of the new antigen with two standard units of the known serum and with .1 c.c. of the selected complement dilution.

The anticomplementary dose is determined by titration of the antigen dilution with complement alone.

As a control of anticomplementary reaction in the serum, titrate four fixation units of the homologous serum with complement alone. This control must show complete hemolysis.

As a control of specificity, titrate the antigen dilution with two fixation units of a heterologous serum and complement. This control must show complete hemolysis.

Fix in the water bath at 37°C. for the required time, and fill with cells which have been sensitized with three standard units of amboceptor.

Standard dilution of the new antigen. For the standard dilution of the new antigen select that dilution in which the fixation unit is .05, the anticomplementary dose .4, or more.

Once standardized, the antigen is always used in that dilution. The dose for tests is .1 c.c. of the standard dilution. This dose contains two fixation units and one-fourth, or less, of the anticomplementary unit.

CULTURE MEDIA FORMULAE.

Veal Agar.

Meat Infusion (young veal)	5 liters.
Peptone	1 per cent.
NaCl	0.5 per cent.
Agar	2 per cent

Melt the agar, cool at 45° C., add one egg per litre. Titrate and make the reaction neutral to phenolphthalein. Autoclave at ten to fifteen pounds pressure for thirty minutes, or place in Arnold sterilizer for one hour. Filter through cotton in a hot filtration funnel. Titrate again, and adjust the reaction to the desired point.

Salt-free-veal-agar. Same as above, leaving out NaCl.

Glycerine-veal-agar. To 95 per cent. of stock veal-agar add 5 per cent. of glycerine. Adjust the reaction to the desired point. Sterilize in autoclave.

Glycerine-veal-horse-serum-agar.

Glycerine-veal-agar (reaction about Ph 7.4 or 0.6 per cent acid to phenolphthalein) 2 parts; sterile normal horse serum 1 part (When sterile ascitic fluid is available, it may be substituted for the horse serum, with the same results). Melt the glycerine-veal-agar, reduce the temperature to 56°C. and add the sterile normal horse serum or ascitic fluid. For slants the proportion of 2 parts agar to 1 part serum gives a good growth; for the antigen plates it is necessary, if the agar is not very firm, to use 3 parts of agar to 1 part of serum.

Coagulated-horse-blood-agar. (So-called "Chocolate Agar.")

Glycerine veal agar (reaction about Ph 7.4 or 0.6 per cent. acid to phenolphthalein) 9 parts; sterile normal horse blood, 3 parts. Melt the agar, and while the medium is hot add the horse blood (whole blood drawn from vein into one per cent. sodium citrate). Make slants and pour plates from this coagulated blood mixture.

Glycerine-Potato-Veal-Agar.

Potato extract 50 c.c. (5 per cent.), glycerine 50 c.c. (5 per cent.), veal-agar (melted) 900 c.c. Adjust to the desired reaction, Ph 5.8—6.0 or 1.6 per cent. acid to phenolphthalein. Sterilize in autoclave.

Potato Extract

Grated potato (white)	1 pound
Water	1 liter

Soak over night, heat to boiling, press through cheesecloth, add one egg per litre. Heat in Arnold one hour to clear. Filter through cotton. Autoclave.

Bordet-Gengou Medium: Water 1000 c.c., glycerine 40 c.c., potatoes (sliced) 500 grams. Heat in an autoclave at 15 pounds pressure for a half-hour. Pour off the liquid. To 500 c.c. of this potato extract add salt solution (0.6 per cent.) 1500 c.c. and agar 60 grams. Autoclave to dissolve, filter and tube. (Reaction after sterilization Ph 7.2 to 7.4). When used, an equal quantity or less of defibrinated rabbit blood is added.

Disodium Phosphate Agar.

Disodium phosphate 0.5 per cent., peptone 2 per cent., agar 2 per cent., bob-veal infusion 1000 c.c. Dissolve over open flame. Titrate, and make the reaction neutral to phenolphthalein. Autoclave thirty minutes at 15 pounds pressure, filter. Titrate. Make reaction about Ph. 7.6 or 0.6 per cent. acid to phenolphthalein.

Dopter's Liver Medium.

Minced fresh calf or beef liver 500 grams. Distilled water 1000 grams. Stir well, and infuse over night in ice-box. Boil five minutes, filter through paper. Make up to original volume by adding water. Add peptone 1 per cent., NaCl 0.5 per cent., agar 1.5 per cent. Boil twenty minutes and adjust to 0.2 per cent. acid to phenolphthalein. Boil five minutes. Filter. Sterilize in Arnold on three successive days. For this medium the liver must be fresh, as cold storage liver gives uncertain results.

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from the

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DR. WILLIAM H. PARK, Director

VOLUME X
1920-1926

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EDITOR'S NOTE

Owing to the lack of appropriation no volume of Collected Studies has been issued from this laboratory since 1919. During the interval the majority of the articles in the present volume have been published elsewhere and with some revision are reprinted here from the various medical journals in which they originally appeared.

With three exceptions, no papers have been included in the present volume which were written later than the end of 1926.

In certain cases, for the sake of brevity and more systematic arrangement, several papers on the same topic have been combined to form one article, and most articles have been abbreviated, with reference to the original source of publication.

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DEATH OF DR. ANTHONY

The death of one of the most valued members of our staff, Dr. Bertha Van Houton Anthony occurred in 1923 from pneumonia.

Because of her marked administrative ability she was put in charge of the Division of Media and Sterilization, a division which she helped to organize and which she conducted successfully up to the time of her death. In connection with this work she was given the office title of Chief of Division.

Dr. Anthony had a remarkable ability to express scientific facts in clear and correct English. For a number of years she assisted in editing the Collected Studies and of late had entire charge of this arduous and exacting task. In doing this work, which required so much time and patience, she rendered an invaluable service to all members of the laboratory staff. We are indebted to Dr. Anthony for the idea of general editorial supervision and in no line of work will she be more keenly missed than in this.

TOXIN-ANTITOXIN IMMUNIZATION AGAINST DIPHTHERIA*

William H. Park, M.D.

The development of antitoxin in the bodies of animals injected with diphtheria toxin is one of the best established of facts.

Unaltered diphtheria toxin has been used also as a stimulating injection in man, but it has to be used in very small and repeated doses; otherwise it would produce annoying local reactions and possibly even paralysis. It was early realized that if we ever attempted actively to immunize children, the advantage would be great if we could replace pure toxin with an altered toxin which would be equally effective and without danger.

Use of Toxin Modified by Antitoxin for Active Immunization in Animals

The earliest knowledge that injections of toxin almost neutralized by antitoxin are capable of stimulating in animals the production of antitoxin came quite accidentally. As is well known, antitoxin can be detected and measured only by its characteristic of neutralizing toxin. The degree of neutralization of the toxin by the antitoxin is determined by the injection of a definite quantity of the mixture into guinea-pigs. The testing of the potency of the drawings of the serum from the various horses under treatment is likely to leave a certain proportion of the test animals alive because of their having received subcutaneously toxin with an overneutralizing amount of antitoxin. The attempt to use these treated guinea-pigs several months later revealed the fact that many of the animals were immune. Investigation proved that this antitoxin immunity did not appreciably develop until the lapse of from four to six weeks.

Babes, in 1895, was the first to inject experimentally diphtheria toxin-antitoxin mixtures and to appreciate that not only slightly underneutralized diphtheria toxin, but also that which was slightly overneutralized, would bring about the development of antitoxin in animals.

A little later (1896), but independently, I made the same observations. Wernicke, in 1895, noted that guinea-pigs actively immunized by the injection of living bacilli and antitoxin gave birth to immune young, and that antitoxin was present in both the mothers and their offspring for at least eight months. In the winter of 1896, I began to use this knowledge concerning much overneutralized toxin practically, in starting the immunization of the horses which were employed for the production of diphtheria antitoxin.

I noted that the use of large amounts of overneutralized toxin was a safe and rapid method of beginning immunization. In the same year, Dreyer and Madsen made systematic investigations as to the effect of different degrees of overneutralization of the effectiveness of the toxin-antitoxin to stimulate antitoxin.

In 1903 I published results showing that an injection into a horse of 10,000 lethal doses of toxin which has been just neutralized usually causes the development of about 60 units of antitoxin per cubic centimeter of serum, while the same toxin neutralized sixfold usually causes the production of about three units.

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*Published in The Journal of The American Medical Association, 1922, Vol. LXXIX, pp. 1584-1590.

In 1905, Theobald Smith studied the duration of immunity in guinea-pigs which had received toxin-antitoxin. He corroborated the work of Wernicke, and extended to two years the time during which guinea-pigs retain immunity.

The fact that the antitoxin would take a number of weeks to develop in those not originally possessing it made it not applicable to persons in immediate danger of infection. Therefore, in the presence of diphtheria, immunizing antitoxin injections would still be necessary in families and institutions. In the absence of any simple test for determining which individuals had natural antitoxin and which did not, we had to inject many unnecessarily, if active immunization were to be attempted. The success of the treatment would also be difficult to determine. These considerations delayed its application in man. On the other hand, we had the growing conviction that, wonderful as were the results of antitoxin, diphtheria could never be conquered by it. The combination of the ever present carrier of bacilli, and the slowness of people to recognize diphtheria when it develops, still permits the occurrence of 50 per cent. more diphtheria than we would otherwise have, and perhaps one sixth of the number of deaths. Furthermore, vital statistics revealed that diphtheria mortally and morbidity had not decreased during the last five years; for instance, in the state of New York, the mortality was 12.8 in 1917 and 16.8 in 1921.

Only one means presented itself to our minds which gave any hope of making another distinct advance. This was active immunization by means of toxin injections.

In 1913, the application by Schick of the intracutaneous neutralization test to detect antitoxin immunity gave a simple means of testing for antitoxic immunity before and after the injections.

Practical Application of Diphtheria Toxin-Antitoxin in Man

Von Behring, May 8, 1913, reported the injections of neutralized toxin in a small number of persons. Before giving the injections he used no Schick tests or other means of testing whether or not the subjects were already immune. He demonstrated by repeated guinea-pig tests that there was a quick development of antitoxin in many of those treated, but in others no antitoxin was detected at the time the tests were made. It is now known that the retests were made too soon to detect the development of antitoxin in those who had none at the time of the injection. The duration of the immunity was not investigated. Although von Behring referred to the toxin-antitoxin mixture as his discovery, what he used was exactly what several of us had described and used in experimental immunization during the last eighteen years. His real contribution was the demonstration in a few human beings of the safety of the injections.

The example of von Behring led Hahn and Summer shortly afterward to offer the toxin-antitoxin to the 4,300 children of six villages in the district of Magdeburg, where diphtheria was endemic. Of the 1,097 children children injected, 633 received the full series of three injections; 225 received two injections, and 209 received one. The Schick test was not used before or after treatment, so it is impossible to know how many of the injected children possessed natural antitoxin and how many of the others developed it.

There was no difference in the development of cases of diphtheria among the treated and the untreated portion during the first two weeks following the completion of the injections; but after that time there was a lessening of the number in the treated portion. The immunization had no apparent effect on freeing carriers from infection. Until after the war, no further observations or immunizations were made in Europe.

Oct. 21, 1920, Bieber published his study of the histories of all the children mentioned above. Fortunately, he was able to trace the majority of those who were living in the district at the time of the injections. He reported that during the six years, 15 per cent. of those who had not received injections had had attacks of diphtheria; that only 4.6 per cent. of the total number inoculated, and only 3.3 per cent. of the fully treated had developed it. Because of the war, many of the diagnoses were not confirmed by cultures, and he believed that some of the cases were almost surely not diphtheria. Two of the incompletely treated children had died from diphtheria.

Bieber concluded that although the toxin-antitoxin had not given a lasting immunity to all who had received the injections, the results were satisfactory in that the percentage of persons having diphtheria was very much less in the inoculated portion than in that uninoculated. He believed that the results should stimulate more extensive trials.

PERSONAL OBSERVATIONS

Before and since the period of the war, the practical value of the toxin-antitoxin injections was subjected to continuous investigation by workers in the Bureau of Laboratories of the Department of Health of the City of New York. The results obtained and the conclusions drawn as follows:

THE ANTITOXIN RESPONSE AND THE PERMANENCE OF THE IMMUNITY ACQUIRED

Late in 1913 we began the practical use of toxin-antitoxin injections for the immunizing of children against diphtheria, and established the facts that the procedure was harmless and that after three injections about 80 per cent. of those individuals possessing no antitoxin or insufficient antitoxin to protect from diphtheria developed immunity. Those showing positive Schick reactions and receiving two injections, developed negative Schick reactions in about 70 per cent.; those receiving one injection, in about 50 per cent. We soon realized that the most important problem was the duration of the antitoxic immunity in those that had developed anti-toxin. A satisfactory answer to this question required that immunizations be carried out in institutions where the children would be under observation for a number of years. A few suitable institutions were immediately sought for and obtained by Dr. Abraham Zingher and, later, additional ones were added by Dr. M. C. Schroeder. We have thus been able to keep under supervision for from three to six and one-half years about 10,000 children. From year to year, Drs. Zingher and Schroeder have been reapplying the Schick test to these original children. With a few of them we are now in the seventh year of observation. We have had no serious immediate or late after-effects. In these institutions diphtheria has not developed in any child who has received three injections. Eighty per cent. of those who received three inoculations have developed sufficient antitoxin within three months to prevent the positive Schick reaction. Fifty per cent. of the remainder developed antitoxic immunity sufficient to give the negative Schick test before the end of the first year. The remainder received then or later a second series of injections, and all of these concerning whom we have information became immune. In some later investigation, we have encountered occasional children who resisted even two series of injections.

DISCUSSION OF RESULTS OF TESTS

As an example of the results following the injections in the school-children, Table 1 is given.

The knowledge that about 80 per cent. of the children who possess no diphtheria antitoxin develop it after three injections of toxin-antitoxin and with very few exceptions retain it for at least six years, and that those who partially fail to respond do respond after a second series of treatments, affords us ground for the belief that we have a practical means of immunizing the child population of the country. Even though the belief that this change in the antitoxin content of these children is a permanent one should prove erroneous, it would merely mean that one would have to repeat the injections not oftener than once in six years.

TABLE 1.

RESULTS OF ATTEMPTED IMMUNIZATION AS SHOWN BY SCHICK TEST THREE OR MORE MONTHS AFTER TREATMENT WITH THREE INJECTIONS OF TOXIN-ANTITOXIN; CHILDREN FROM PUBLIC SCHOOLS OF BROOKLYN; TESTS BY DR. SCHROEDER

Number of School	Location	Total Number Children Retested	Positive Cases Which Became Immune	Per Cent.	Length of Period Between Treatment and Retest
158	Belmont Ave. near Warwick St...	370	254	68.7	3 mos.
156	Sutter Ave. near Barrett St......	329	292	88.8	3 mos.
173	Penn. Ave. near Liberty Ave.....	163	144	88.9	4 mos.
29	Columbia and Amity Sts..........	57	45	79.0	5 mos.
142	Henry and Rapelye Sts...........	127	112	88.0	5 mos.
50	S. 3d St. and Driggs Ave.........	82	74	90.3	6 mos.
16	Wilson Ave. near Bedford Ave...	241	216	89.7	6 mos.
72	New Lots Road.................	199	195	98.0	6 mos.
109	Dumont Ave. near Sackman St...	141	99	70.0	6 mos.
4	Berkeley Pl. near 5th Ave........	103	91	88.4	7 mos.
	Total	1,812	1,522	83.9	

PERMANENCE OF NEGATIVE SCHICK REACTION IN PERSONS WHO DEVELOP NATURAL IMMUNITY

At the Convent of St. Dominick, ninety-four of the original children who had given Schick negative reactions with Dr. Zingher's tests remained for five years. These children were retested at the two and five year periods by Dr. Schroeder. In both retests ninety of these children showed negative reactions, while four showed positive reactions in one or the other retest. It is an interesting point as to whether the children who were negative originally had lost their antitoxin or whether the apparent change in reaction was due to other causes. We know from making the double Schick test (that is, one test on each arm) on several hundreds of children, that routine tests made even by an expert are likely to show occasional errors. In this test series, about 2 per cent. of the children showing positive tests had them only on one arm. There was, therefore, no doubt that in 2 per cent. of these children, one of the two injections had been inserted too deeply so that the toxin did not remain in the skin and therefore could not produce the reaction. We know also that slight differences in the strength of the toxin solution cause a borderland case to give either a negative or a positive reaction. Differences in technic and in toxin solution possibly account, therefore, for the apparent change in one or all of the four cases. On the other hand, we know that the amount of antitoxin in an individual changes somewhat from time to time, so that it is possible that one or all of the four subjects might have reacted at one time and not at another to the standard Schick toxin properly given.

Whatever the explanation of the apparent change in four cases, we have the remarkable fact that nearly 96 per cent. of the originally negative children remained negative during five years. Practically the same results have been obtained in all the other institutions.

INFLUENCE OF AGE ON SUSCEPTIBILITY AND THE RESPONSE TO IMMUNIZING INJECTIONS

It is common belief that the mortality from diphtheria is greatest at the ages of from one-half year to 4 years, inclusive; that it then drops steadily until at 10 years it is quite low and so remains during the rest of life. The figures in Table 2 give evidence that this belief is founded on fact.

TABLE 2.

DEATHS FROM DIPHTHERIA GROUPED BY SEX AND AGES, CITY OF NEW YORK, YEAR 1917

Age	Males	Females	Total	Per Cent.
Under 1 year	73	60	133	11+
1 to 4 years inclusive	390	319	709	60+
Total under 5 years	463	379	842	72+
5 to 9 years inclusive	119	132	251	21+
10 to 14 years inclusive	9	13	22	2
15 to 19 years inclusive	6	4	10	1
20 to 24 years inclusive	2	8	10	1
25 to 60 years inclusive	9	14	23	2
Total all ages	608	550	1,158	

The greater liability to infection during the first years of life is clearly shown in the results from the Schick test. Our findings in New York City are given in Table 3.

All reports show that under similar conditions the highest percentage of persons having antitoxin occurs among infants passively carrying their mothers' antitoxin, and among adults; between the ages there is first a decrease and then an increase. The same reports show that similar age groups of different children reveal surprising differences in the percentages of those possessing antitoxic immunity.

TABLE 3.

AVERAGE SUSCEPTIBILITY OF VARIOUS AGES TO DIPHTHERIA (AS INDICATED BY THE POSITIVE SCHICK DIPHTHERIA-TOXIN SKIN TEST), IN NEW YORK CITY*

Age	Schick + (Susceptible)
Under 3 months	15 per cent.
3 to 6 months	30 per cent.
6 months to 1 year	60 per cent.
1 to 2 years	60 per cent.
2 to 3 years	60 per cent.
3 to 5 years	40 per cent.
5 to 10 years	35 per cent.
10 to 20 years	25 per cent.
20 to 40 years	18 per cent.
Over 40 years	12 per cent.

*The percentage of positive reaction is considerably higher among those living in the country.

Table 4 notes the very small percentage of susceptibles in an institution caring for little children gathered from the poorest and most crowded homes of the city. Table 5 exhibits the surprising differences in the results obtained from tests of pupils in different parts of Brooklyn.

TABLE 4.

RESULTS OF THE SCHICK TEST IN AN INSTITUTION CARING FOR CHILDREN GATHERED FROM DENSELY POPULATED PORTIONS OF NEW YORK CITY

	Shick Reactions				
	Positive		Borderland	Negative	
Age	+	±	±	—	Per Cent. Positive
Under 5 years	6	2	0	35	18.5
5 to 10 years	21	2	8	220	9.0
10 to 15 years	5	2	4	79	7.7
	32	6	12	334	9.9

The sign ± indicates a trace of antitoxin.

A minimum, an average and a maximum result from among the larger Brooklyn public schools subjected to the Schick test by Dr. Schroeder or her assistants is given in Table 5. The ages ranged from 6 to 12 years.

TABLE 5.

DIFFERENT PERCENTAGES OF CHILDREN GIVING SCHICK-POSITIVE REACTIONS IN FOUR LARGE PUBLIC SCHOOLS

No. of Brooklyn School	Total Number of Tests	Per Cent. Positive
50	1,433	19.60
125	1,064	36.84
149	1,056	41.19
156	1,148	61.84

The highest percentage of positive tests obtained by Dr. Schroeder in any Brooklyn school was 72, and the lowest 16. These were in small schools. The average percentage of positives among 30,000 children in seventy-three Brooklyn schools was 41.5.

In the Manhattan schools at the same time or a little later, Dr. Zingher among 52,000 children found that the percentage of positives was 30. The highest percentage of positives in any school was 67, and the lowest, 13.6.

The results obtained by Dr. Schroeder among adults are very interesting as showing a much older age group. Among 4,396 adult inmates in an insane asylum there were 633 positive reactions, a percentage of 16. At all ages between 20 and 50 years, the percentage was about the same, but above 50 years of age the positives dropped to 10. There were a good many persisting pseudoreactions which made it extremely difficult in some instances to decide between a pseudoreaction and a combined reaction. The patients responded to the toxin-antitoxin injections as well as younger people, except that they showed more local reaction.

IMMUNIZING EFFECT OF TOXIN-ANTITOXIN INJECTIONS IN INFANTS AT BIRTH AND DURING THE FIRST TWO YEARS OF LIFE

If it were possible to immunize young infants, this would be most desirable. In order to test this possibility, 2,000 infants were given full doses by Miss Denny on the third, eighth and eleventh days after birth. Most careful observations revealed absolutely no deleterious effects. At the end of a year, 100 of these infants were retested. Only 52 per cent. gave negative Schick reactions. Since untreated infants of this age give about the same result, it is evident that the combined effect of immature cells and the overneutralization of the toxin-antitoxin present (because of the passive immunity derived from the mother) prevents any appreciable response at birth to the toxin-antitoxin injections. Infants aged 6 months and over gave far better results.

Thus Dr. Blum observed the results of the injections in a number of older infants, in the Home for Hebrew Infants. His figures are given in Tables 6 and 7.

Dr. Byard also reports very favorable results among children in private homes. Many of these were not given the Schick test before receiving the immunizing injections. It is fair to assume that about 50 per cent. would have given negative Schick reactions. Of 286 such children, more than half

TABLE 6.

RESULTS OF TOXIN-ANTITOXIN INJECTIONS IN SCHICK-POSITIVE CHILDREN

Number of Infants	Age	Toxin-Antitoxin Injections	Lapsed Period at Time of Retest	Results of Shick Test
6	5 mos.	3	3-5 mos.	—
5	5 mos.	3	9 mos.	—
3	6 mos.	3	4 mos.	—
4	7 mos.	3	4-5 mos.	—
12	8 mos.	3	4-5 mos.	—
Total 30				

TABLE 7.
RESULTS OF TOXIN-ANTITOXIN INJECTIONS IN SCHICK-NEGATIVE INFANTS

Number of Infants	Age	Toxin-Antitoxin Injections	Lapsed Period at Time of Retest	Results of Schick Test
1	3 mos.	3	4 mos.	±
2	4 mos.	3	4 mos.	—
2	5 mos.	3	4 mos.	—
3	6 mos.	3	4 mos.	—
6	7 mos.	3	4 mos.	—
1	8 mos.	3	4 mos.	—
1	9 mos.	3	4 mos.	—
3	10 mos	3	4 mos.	—
1	11 mos.	3	4 mos.	—
Total 20				

of whom were under 1 year of age when injected, he noted that 143 (under 2 years of age when injected) when retested after seven months showed 136 negative, five positive and two very doubtful Schick reactions. Of the whole number, seven, or 2.4 per cent., when retested at the end of eight months were definitely positive. Eighteen months after the injections, 1.8 per cent. were positive. These results are certainly very encouraging.

These results of Blum and Byard among infants aged 4 months and over are of extreme practical importance, because from 6 months to 3 years is the period when immunization is most necessary and when it creates the least disturbance.

Constitutional and Local Reactions Following the Toxin-Antitoxin Injections

Constitutional and local reactions following the toxin-antitoxin injections are negligible in the infant; slight and infrequent in the young child; moderate or rather severe in perhaps 10 per cent. of older children, and slight, moderate or quite severe in a large percentage of adults. The effects are largely due to the protein contents of the culture fluid and are not due to the toxin as such. This is evident because the same reaction follows the injection of the toxin broth rendered atoxic by heating, or of a solution containing a minute quantity of autolyzed diphtheria bacilli. If the toxin were the cause, there would be little or no reaction in naturally immune persons. As is well known, these show fully as much reaction as those who have no antitoxin, if not more reaction. Those individuals who give the strong pseudoreactions with the heated or unheated toxin of the Schick test are those who give the most severe reactions with the toxin-antitoxin injections. However, some who give no pseudoreaction with the Schick test give moderately severe reactions to the toxin-antitoxin injections. The horse serum is present in such a minute amount as to cause no appreciable reaction except in a few extremely susceptible individuals. It does not seem to sensitize them appreciably to later doses of horse serum.

In children of school age about 10 per cent. develop fairly sore arms and temperature of from 99 to 103 F. About 5 per cent. feel miserable enough to stay at home from school for one day, and a very few for two days. We have given about 40,000 inoculations this fall and winter without a single infection. Children that are constipated are advised to take a laxative on the day of the injection and to apply a moist dressing to the arm if swelling and soreness develop.

In adults, the reactions are not quite as severe as with the typhoid inoculations. The most severe reactions are restricted almost entirely to those who develop the marked pseudoreaction with the heated or overneutralized toxin. The following history gives an account of one of the most severe reactions that we have encountered:

A nurse at the Willard Parker Hospital was given toxin-antitoxin subcutaneously in the right arm, February 8. In the evening the arm in the region of the injection felt sore and looked red. The next day the soreness and tenderness were more marked. The highest temperature was 103 F. February 9, the patient was in bed. The temperature was 99. The right arm showed moderate redness of the lower two thirds, some induration and tenderness, and slight axillary tenderness. February 10, constitutional symptoms were absent. The temperature was normal. Redness and swelling persisted; induration was moderate; tenderness and pain were less marked; axillary tenderness was very slight. A Schick test and control which were made, February 9, both showed a moderate pseudoreaction, the two reactions appearing alike. An area of redness about the size of a 25 cent piece and some induration were present. A small secondary areola around the central area was of marked redness. The probability is great that the test was a pseudoreaction and not a combined reaction.

THE TOXIN-ANTITOXIN MIXTURE

The best mixture is one that is underneutralized and yet perfectly safe. As the mixture stands, the toxin deteriorates faster than the antitoxin, so that it gradually becomes neutralized and later overneutralized by the antitoxin.

TABLE 8.

RESULTS OF INJECTION OF DIFFERENT PREPARATIONS OF TOXIN-ANTITOXIN IN GUINEA-PIGS

Preparation										
Number	Amt. in C.c.	Number Doses	Weeks Elapsed	Percentage of Neg. Schick Reactions	Number Doses	Weeks Elapsed	Percentage of Neg. Schick Reactions	Number Doses	Weeks Elapsed	Percentage of Neg. Schick Reactions
24	0.5	1	7	100	2	5	100	3	7	100
18	0.5	1	12	66	2	4	75	3	9	100
22	0.5	1	8	50	2	8	50	3	7	100
20	0.5	1	..	..	2	9	33	3	9	25
24A*	0.5	1	..	..	2	7	50	3	7	50
24	0.1	3	7	75	4	7	100			
18	0.1	3	10	50	4	9	60			
22	0.1	3	8	25	4	8	25			
20	0.1	3	8	0	4	8	40			
24A*	0.1	3	7	0	4	8	0			
10	0.1	3	8	0	4	8	0			

*Preparation of 24 with an added 10 per cent. of its original amount of antitoxin.

Therefore, a safe preparation is at its best when freshly prepared. As it would not be practicable to make new mixtures every day, we keep both the toxin and the antitoxin until they have become stabilized. After this has occurred, the toxin-antitoxin mixture changes very slowly. Even so, it is better not to use mixtures later than three months after their preparation unless animal tests prove them to be still slightly underneutralized.

It is noticeable that Preparation 24 gave much better immunizing than any of the others, except Preparation 18, which was a little inferior. The addition of 10 per cent. of the original amount of antitoxin to Preparation 24 lessened greatly its value. The other preparations had stood for some time, become less toxic and lost in immunizing power.

The comparative results of animal and human inoculations with different preparations show that very considerable differences in immunizing value exist. As yet we find it extremely difficult to determine by animal tests the finer differences which decide just when the ideal degree of neutralization is obtained. Dr. Banzhaf now standardizes our preparation so that 1 c.c. causes paralysis in the majority of guinea-pigs weighing 250 gm.; in all, 5 c.c. causes paralysis which ends in death. The results obtained in children and in animals with five different preparations clearly bring out the different degrees of success which are obtained with unequally neutralized preparations (Tables 8 and 9).

TABLE 9.

IMMUNIZING EFFICIENCY OF DIFFERENT MIXTURES OF TOXIN-ANTITOXIN IN CHILDREN

School or Institution	Mixture Three Doses 1 C.c. Each	Date Injected	Date Retested	Total Number of Children	Results +	Results —	Percentage of Positive Reactions
Colored Orphan Asylum	No. 18	12/ 5/19	3/ 1/20	70	3	67	95.7
Hebrew Sheltering and Guardian Home	No. 22	4/24/20	10/23/20	84	27	57	70.2
N. Y. Orphanage	No. 22	6/ 5/20	10/23/20	63	35	28	44.4
P. S. No. 90.....	No. 24	12/17/20	5/ 3/21	160	20	140	87.5

Safety of Toxin-Antitoxin Injections

In utilizing a substance which is a combination of a poison and an antipoison, it is of extreme importance to be certain that there is no possibility of a properly neutralized mixture becoming at some later period toxic. We have tested out many mixtures and kept them at all possible temperatures, and always found that the mixtures became very slowly more and more overneutralized. The antitoxin portion of the combination is more stable than the toxin. The only objection to using products that have been kept a long time after neutralization is that they are somewhat less effective. The only accident that has occurred in the use of toxin-antitoxin mixtures, so far as is known, was due to an error through which an improperly tested product was sent out. The regulations now adopted by all biologic plants make a repetition of such an accident impossible.

PRACTICAL APPLICATION IN THE PUBLIC SCHOOLS

The health and school authorities determined to put their chief efforts for the first two years into an attempt to immunize as many schoolchildren as possible rather than endeavor to protect the children of preschool age who needed the protection so much more. The reasons for this were many and important. It was deemed wise to acquaint as many parents and others as possible with the value of the Schick test and the toxin-antitoxin injections. No better way seemed available than to use the schools as the means of doing this. If each pupil presented his parents with a circular picturing the danger from diphtheria, describing the preventive treatment and asking for permission to administer this treatment if the family physician approved, it would mean that nearly a million adults and a million children would have the arguments for the use of the toxin-antitoxin vaccine presented to them in a favorable way. If, as we hoped, about one half of the children brought back the acceptance of the offer to give them the toxin-antitoxin, we should be able not only to immunize those shown by the Schick test to possess no antitoxin but, by preventing those children from contracting diphtheria, we should also prevent their carrying diphtheria home to the younger children in their families.

The testing of the schoolchildren would also give us the chance to determine exactly what effect the immunization had had. We could make an index of 90,000 children who had been tested and, when Schick positive, had been injected with toxin-antitoxin; a similar index of 90,000 children of the same ages who had refused the test and the injections could be used for comparison. The cases of diphtheria occurring among the children of school age could thus be looked up in either index, and observations made as to how many cases of diphtheria occurred in the two groups. We believed that after one or two years of such observations we should have had evidence of convincing value as to the protection guaranteed by a negative Schick test due either to natural antitoxic immunity or to the response to the toxin-antitoxin injections.

Now, at the end of two years, we actually have such an index completed. The 90,000 tested children and the 90,000 control children have been gathered about equally from the schools of the different boroughs; they were collected by the groups of nurses and teachers from the schools which had been tested by Dr. M. C. Schroeder and Dr. Abraham Zingher. Among an equal number of untreated and treated children, there were four times as many cases among the untreated children. The majority of the cases in both groups were of slight or moderate severity. Some of them were possibly cases of nondiphtheritic tonsillitis in children who happened to be carriers of diphtheria bacilli. The methods which we have adopted in the diphtheria prevention work in the schools have already been published by Dr. Zingher (No. 96 of the Reprint Series, Department of Health, City of New York). I will describe them briefly.

A very important point is the enlistment of the interest of the principal of a school. As a rule Dr. I. H. Goldberger, of the bureau of educational hygiene of the department of education, first prepares the way by obtaining permission for us to do the work in the school. According to the borough in which the school is situated, Dr. Zingher, Dr. Schroeder or one of their representatives sees the principal and explains fully the objects we have in view. Literature is left for the teachers. Either the principal or the physician meets the teachers in a conference, gives them the necessary information, and tries to arouse their enthusiasm. The success or failure in getting a favorable response from the children or their parents depends largely on the interest which the principal, the assistant principal and the teachers take in the matter. When they give us their enthusiastic cooperation, we expect to obtain consents from three fourths of the parents; when we fail to arouse the teachers' interest, we are fortunate if this is obtained from one fourth of them.

The preparatory work being furnished and the date for the test being determined, we send to the school the circulars and consent slips to be taken by the children to their homes.

Table 10 shows the results among the 180,000 schoolchildren whom we have listed.

TABLE 10.

NUMBER OF CASES OF SUSPECTED DIPHTHERIA DEVELOPING IN 90,000 UNTREATED SCHOOLCHLDREN AND IN 90,000 TESTED BY SCHICK METHOD AND WHEN POSITIVE GIVEN TWO OR THREE INJECTIONS OF TOXIN-ANTITOXIN

	Cases Reported as Clinical Diphtheria
In Brooklyn:	
26,000 originally Schick-negative children (observation from October 1 to February 15)	2
15,000 Schick-positive children, 3, 2 or 1 toxin-antitoxin injections	4*
40,000 control children of same ages	27
In Manhattan:	
31,000 Schick-negative children (observation from October 1 to February 15)	3
19,000 Schick-positive children, 3, 2 or in injections	5
50,000 control children	29
Summary:	
57,000 Schick-negative children (observation from October 1 to February 15)	5
33,000 Schick-positive children injected with toxin-antitoxin	9
Among a total of 90,000 Schick-negative or injected children	14
Among a total of 90,000 control children untreated	56

*Two of these children received but one injection and had no Schick retest. The third showed a Schick + and the fourth a Schick ± reaction on the retest and refused further injections.

As may be seen, there were among the 90,000 untreated schoolchildren four times as many cases of probable diphtheria as developed in the 90,000 tested children.

Summary and Conclusions

The results of twenty-five years of experimental and practical investigation of the immunizing effect of toxin-antitoxin injections and the value of the Schick test are as follows:

Three injections, 1 c.c. each, of a suitable toxin-antitoxin mixture spaced one or two weeks apart, will cause about 85 per cent. of susceptible children or older persons to develop sufficient antitoxin to give the negative Schick reaction and produce marked, if not absolute, protection against diphtheria.

The development of the immunity is slow. An amount of antitoxin sufficient to prevent the positive Schick reaction develops in the different children in from one to six months after the receiving of the injections. Antitoxin as heretofore must continue to be used to produce immediate immunity.

The duration of the immunity in at least 90 per cent. of the children is for more than six years and probably for the remainder of life. There seems to be no difference in this respect between these, and those who develop antitoxin naturally.

Toxin-antitoxin injections should not be given within two weeks after an injection of antitoxin; otherwise the toxin is slightly overneutralized and the resulting development of antitoxin is lessened.

Mixtures made from old toxin and antitoxin are fairly stable and may be used for a period of one year. Each such preparations are at their best when first sent out, as the mixtures slowly tend to become at first neutralized and then slightly antitoxic. This change gradually lessens the immunizing power of the toxin. The toxin-antitoxin should be kept cool and in a dark place; it is best to use the mixtures within three months after their final preparation.

A toxin-antitoxin mixture of stabilized materials which is safe when it leaves the laboratory cannot become more toxic on being kept. No serious effects have ever resulted from the injections given to the tens of thousands of the New York children since we began our work, seven years ago.

The Schick test is an extremely reliable means of separating those individuals who have antitoxic immunity from those that have none. Although a simple test, it must be carried out with extreme care. The toxin must be retained intracutaneously, and the toxin must be neither 25 per cent. more nor less than the desired amount. It is extremely important to choose glass of suitable chemical composition for the containers in which the toxin is to be placed, as otherwise rapid deterioration may take place.

The preliminary Schick test is usually omitted in children under 3 years of age. This is for two reasons: 1. Two thirds of these children require the toxin-antitoxin injections anyway. 2. We are not certain whether those that do give the negative reaction are immune because of an unusual persistence of the antitoxin given them by their mothers, or because of the active development of antitoxin in their own bodies. After this age the test is desirable, but it is often omitted.

Thus, in practical school work, the first Schick test is frequently omitted in children up to the age of 6 because it is easier to inject the children at once rather than to delay for the test. At this age the percentage of children requiring immunization is still high, and the annoyance from the injections is slight. The omission of the preliminary Schick test facilitates the introduction of the immunizing injections in the schools. Above the age of 6 years, the preliminary Schick test should be made whenever practicable.

No child should be pronounced immune from diphtheria because of having received three immunizing injections of toxin-antitoxin. A negative Schick test is absolutely necessary before one can properly make such a statement or issue a certificate.

The use of the control protein test made with the heated toxin is advisable at all ages when a careful separation of the pseudonegative reaction from the combined positive reaction is important. As with the Schick test, it is frequently omitted because of local conditions. The older the child the more likely it is to be immune and to give a confusing protein reaction.

In children under 5 years of age, the protein reaction seldom confuses the picture if the Schick tests are read as late as on the fourth day; between 5 and 7 years, the control does not help greatly in more than 5 per cent. In older children and adults, not only does the control protein test help us to decide more correctly in about 10 per cent. of doubtful reactions; but when it is marked it also indicates with some probability those persons who are likely to have the marked local and constitutional reactions from the toxin-antitoxin injections.

The toxin-antitoxin injections are inadvisable before the age of 6 months. During this time most of the infants retain the antitoxin received from their mothers. Up to the age of 3 months, immunizing injections are usually ineffective, as the infant tissues do not respond sufficiently during this period to the toxin-antitoxin to produce antitoxin. Under usual conditions, it is probably safe to wait until the infant is 9 months old and then to give the injections at the first suitable occasion. During the first three years there is almost no annoyance from the injections. As the child grows older, the danger from diphtheria gradually lessens, and the percentage of those developing annoying local and constitutional reactions slowly increases.

The immunization of schoolchildren in acting to prevent their contracting diphtheria also lessens the exposure to infection of the younger children of preschool age in their families.

There appears to be no difference in the degree of immunity between those individuals who have developed antitoxin from natural causes and those who did so because of the stimulus of the toxin-antitoxin injections.

Institutions in which the children have been given the immunizing injections have been remarkably free from diphtheria.

The schoolchildren who have been injected have had one-fourth as many cases as the untreated children, and these cases have been of less severity.

THE DEGREE OF IMMUNITY TO DIPHTHERIA INSURED BY A NEGATIVE SCHICK TEST

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William H. Park

This communication was suggested by reading an article on an outbreak of tonsillitis suspected to be diphtheritic by Dr. S. A. Blauner.[1] Through the courtesy of Dr. Blauner, I was enabled to study the outbreak described. I saw the last three cases in the acute stage, and made cultures from the sick and convalescent children and from many of the healthy children distributed in the different parts of the institution. The laboratory and clinical material considered together lead me to somewhat different conclusions from those drawn by Dr. Blauner. Although the results of all the laboratory examinations were sent to him, he either failed to remember them or did not interpret them as I do.

Before considering this special outbreak, it seems desirable to state the evidence which supports the view that sufficient antitoxin in the body to prevent the development of a positive Schick test under ordinary conditions will prevent the development of diphtheria. There can be no doubt that the injection of 500 units of antitoxin will give children an immunity for a period of at least twelve days and usually twenty days. The proof of this is derived from its use in many outbreaks and in the exposed children of hundreds of thousands of families. The recent statement by Haven's that about 20 per cent. of diphtheria bacilli are of a different type from the one used to produce toxin for the immunization of horses, and that the available monovalent antitoxin will not completely neutralize the toxin made by this other type, and that, therefore, diphtheria may develop from infection with this other type in children showing a negative Schick test to the standard toxin, has not been borne out by experience nor confirmed by us in experimental work. The evidence that antitoxin is the only substance in the body that can prevent the toxin acting is also so conclusive that we can assume that a negative Schick test means the presence of sufficient antitoxin to neutralize it. The debatable points are: is the minimum amount of antitoxin required to prevent the Schick test sufficient to prevent the development of diphtheria and, if not, is it sufficient to prevent a toxemia. Closely bound up with the answer to these questions is the degree of certainty that the general practitioner can assume that, as ordinarily carried out, a negative Schick test proves the presence of at least this minimum amount of antitoxin. It will be wise to dispose of the last query first.

A Schick test requires the injection intracutaneously of one-fiftieth of a lethal dose for a 250 gm. guinea-pig in 0.1 c.c. or an equivalent amount of toxin in a different amount of fluid. We recommend one-fortieth M. L. D. in 0.2 c.c. because we find the larger amount of fluid easier to give and, therefore, safer for the inexpert. Reports sent us some time ago of several series of cases with no positive Schick reactions caused us to obtain outfits from various manufacturers. Dr. Zingher found in a considerable number of instances that the available quantity of toxin in some of the outfits was much

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1. Am. J. Dis. Child. 21:472 (May) 1921.

less than the required amounts. The children tested with these solutions gave negative Schick reactions when control tests made with standardized toxins gave positive ones. More recent tests of outfits from these firms have given uniformly good results.

Not only must the toxin be measured accurately, but the toxin must be delivered wholly intracutaneously. If injected a little too deeply it will not be retained in the skin and will not exert the desired effect. If the small white area does not appear at the point of injection the Schick test cannot be relied upon. Careful investigation leads us to believe that from 2 to 4 per cent. of the persons giving negative Schick tests do so because of error in technic. When great care has been taken to have the toxin strength correct and the injection given strictly intracutaneously, we have not discovered any errors. Too strong a toxin will give a larger percentage of positives than will the standard amount. The endeavor of Schick was to choose the amount that would as nearly as possible divide the susceptible from the immune.

During the past seven years my colleagues in the laboratory have made over 150,000 Schick tests, and have immunized about forty thousand of those giving a positive reaction. We have also tested from year to year about fifteen thousand children who are inmates in institutions, so as to note any changes. There has been an average difference of about 2 per cent. in the annual readings. This we have considered as partly due to borderline cases and partly to errors in technic. The major part of this work has been directly carried out or has been supervised by Dr. A. Zingher and Dr. M. C. Schroder. We have during these years earnestly sought to discover all cases of suspected diphtheria which occurred among those who had given negative Schick reactions, either before or after the toxin-antitoxin injections.

During this time we have learned of and investigated eighteen cases of suspected diphtheria in those who had given a negative Schick reaction. Two of these were in an institution in Brooklyn, two in an institution in New York, eight in Dr. Blauner's institution and six in their homes. In none of these cases has death occurred. Undoubtedly, there have been other cases concerning which information has not reached us. We are now cataloging 100,000 New York City school children who have given negative Schick tests, and 100,000 untested children from the same schools.

During the next year we are going to look up every case reported as diphtheria and determine the difference between the incidence of diphtheria in the two groups of children. In as far as possible, we will inquire into each case so as to have both the physician's original report and the results of the inspector's investigation. We hope that, by this method, we may get a decision within a year as to the degree of immunity from diphtheria insured to children giving a negative Schick reaction.

It is very difficult to decide whether any of the sixteen cases with positive cultures, having patches of pseudomembrane either confined to the tonsils or spreading to the pharyngeal borders, were cases in which a faulty Schick test had been made, or whether they must be considered moderate cases of diphtheria developing in spite of an amount of antitoxin sufficient to prevent a positive Schick test, or whether they were due to other microbes, the diphtheria bacilli being present by accident and taking no active part. An important fact to remember is that, whatever the final decision is as to the etiology of these cases, they are extremely rare.

A detailed discussion of the outbreak that occurred in Dr. Blauner's service will show the complexity of the factors involved. We will combine a consideration of the clinical observations supplied by Dr. Blauner and the laboratory findings of Miss Mann who carried out extensive investigations under the general direction of Dr. A. W. Williams and myself.

The clinical facts can be stated briefly as follows: In a ward of about twenty children ranging from 2 to 5 years of age, all but one of whom had given a negative Schick reaction, three cases of croupous tonsillitis developed December 23, one on the twenty-sixth, two on the twenty-eighth, one on the twenty-ninth and two on the thirty-first. Eight of these showed grayish patches on the tonsils. In five, the patches spread from the tonsils to the edges of the phraynx. One of the two cases developing on the thirty-first was mild and had no exudate. The temperature of the cases ranged from 102 to 104 F. on the first day, and dropped rapidly by lysis.

Dr. Blauner states in his article that at first he had no suspicion that the earliest three cases had diphtheria because of the previous negative Schick reactions, but when the positive laboratory reports came in he became convinced that they were cases of true diphtheria. The three cases I saw would have been considered by me as probably, but not at all certainly, diphtheria. The cultures from all the cases, except the ninth, which was very mild, contained virulent diphtheria bacilli. The mild case contained no diphtheria bacilli.

Four of the eight moderately severe cases had given negative Schick reactions on their entrance into the institution; three had become negative after injections, and one, though injected, had remained positive. The first seven[2] of the children received antitoxin and recovered promptly; the last two received no antitoxin and recovered equally rapidly. The possibilities seemed to be (1), that the amount of antitoxin as shown by the negative Schick reaction had been in these cases insufficient to prevent moderate diphtheria; (2) that some other infection had caused the croupous tonsillitis, and that the diphtheria bacilli were accidentally present in carriers, and (3) that the sore throat started by the pyogenic organisms had paved the way for the local action of the accompanying diphtheria bacilli. To test out these possibilities we omitted giving antitoxin in the last two cases which had just developed and one of which was very mild. These two cases progressed favorably as I felt certain they would and the children recovered as promptly as did those that had received antitoxin. This result suggested that whether or not the diphtheria bacilli had taken part in the formation of the exudate, the recovery was probably not due to the additional antitoxin injected.

A week later, January 7, cultures were made from twenty-seven of the children in the room in which the infected cases occurred, and from eleven of them virulent diphtheria bacilli were obtained. As these children had been given an immunizing injection of antitoxin, Dr. Blauner thought that the cessation of the cases, in spite of the presence of so many carriers, was a strong point in favor of the former amount of antitoxin having been insufficient to protect. This led me to make cultures from nineteen children taken at random from other parts of the building where no antitoxin immunization had been given. To our surprise, we found the carriers even more abundant here than in the ward from which the cases came. Miss Mann isolated diphtheria bacilli from the cultures made from ten of nineteen children (See accompanying table). All strains were tested and found to be toxin producers. The children in the general building were, therefore, apparently protected by the amount of antitoxin which had given a negative Schick reaction. With these results before us, we see that if the tonsillitis in the ward had been caused by some other infection than diphtheria, the throats, if not already infected, would almost necessarily have become carriers of the bacilli. Dr. Blauner's complete dependence on the culture results to solve the nature of the infection is not surprising, since health departments have so emphasized the value of the bacteriological reports in suspected cases of diphtheria that many physicians believe that a positive report from a suspected case proves that the case is one of diphtheria.

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[2] There is some confusion as to whether it was the eighth case or an additional case that received no antitoxin. There is no doubt that there was a case with tonsillar exudate similar to the eighth case.

The absence of diphtheria bacilli in a culture properly made is fairly good proof that no such bacilli are in the throat and, that, therefore, the case is not one of diphtheria. If diphtheria bacilli are present, the probability of diphtheria being present is greater or less according to the more or less characteristic appearance of the lesions. The laboratory indicates the presence or absence of the bacilli and the danger of the case spreading infection; the clinical examination determines the probability or certainty of the presence of diphtheria. It would be more helpful if the laboratory could make the diagnosis, but, unfortunately, it cannot. If we apply the above facts to Dr. Blauner's cases, we see that, as certainly half of the children in the institution were carrying virulent diphtheria bacilli in their throats during the month of January, whatever nasal or throat infection took place diphtheria bacilli would probably be associated with it. With 50 per cent. of the normal cases carrying bacilli, practically every diseased child would almost immediately become a carrier.

RESULTS OF THE CULTURE EXAMINATION OF NINETEEN HEALTHY CHILDREN FROM WARDS IN WHICH NO DIPHTHERIA HAD OCCURRED

Name	Result	Name	Result
A. R.	Negative	L. E.	Diphtheria bacilli
A. M.	Diphtheria bacilli	L. M.	Diphtheria bacilli
B. F.	Diphtheria bacilli	M. L.	Diphtheria bacilli
B. A.	Diphtheria bacilli	R. L.	Negative
B. W.	Negative	S. L.	Negative
D. M.	Diphtheria bacilli	S. S.	Diphtheria bacilli
G. L	Negative	W. S.	Negative
H. M.	Negative	W. J.	Diphtheria bacilli
H. D.	Diphtheria bacilli	Z. J.	Negative
H. M.	Negative		

Conclusions

A negative Schick test in cases which have an active immunity, either natural or acquired, when the toxin used and technic employed have been suitable indicates an almost complete security from diphtheritic disease, not only for the immediate time but for the future.

Those that have natural antitoxin and those who acquire it through toxin-antitoxin injections may harbor diphtheria bacilli, and if they later suffer from tonsillitis, due to other microbes, throat cultures will contain diphtheria bacilli. The positive culture alone suggests but does not establish that the suspected case is one of diphtheria.

Under unusual conditions it is possible that, when diphtheria bacilli are present in a throat which becomes the seat of some other infection, they may develop their toxin and cause superficial lesions in the mucous membrane even though the cases have sufficient antitoxin to give a negative Schick reaction. Cases which present this possibility are rare, and when met with by us have done well without additional injection of antitoxin.

In the outbreak discussed in this communication, the fact that, while an extraordinarily large percentage of all the children in the institution were carriers of virulent bacilli only one group develop cases of tonsillitis with pseudomembranous patches, makes it uncertain whether the cases were diphtheria.

THE DURATION OF THE IMMUNITY CONFERRED BY THE USE OF DIPHTHERIA TOXIN-ANTITOXIN

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By M. C. Schroeder

The practical value of the toxin-antitoxin immunizing injections depends on several factors: the harmlessness of the injections, the proportion of the susceptibles who respond and become immune and finally the duration of the immunity. Active immunization would have been attempted on a large scale years ago except for the difficulty of identifying those who required protection.

For twenty-five years it has been known that it was possible to immunize with the toxin-antitoxin. In fact in our own laboratory we learned this in 1896 and used the method practically in horses. The suggestion of Schick that the introduction of a minute quantity of toxin in the skin would differentiate those that had antitoxin immunity from those that had none, made it possible to inject those who had no antitoxin and watch the results. This led Behring to undertake active immunization in Germany nine or ten years ago. His success encouraged us to undertake the same work in New York. After we had determined the harmlessness of the mixture and the percentage of those becoming immune after one, two and three injections (see table), we realized that the all-important fact which remained to be discovered was the duration of the immunity. Even if we could make every child immune this would be of little practical value unless the condition remained permanent. We decided that the most favorable means of determining the truth as to this point was to seek institutions where the children were held for a number of years. Dr. Zingher and the writer picked out a number of institutions and we have watched the children from year to year. Five and one-half years have elapsed since the children in the first institution were treated. It is difficult to wait with patience for the years to pass to reach the final conclusion, but there is no other way. We have also tested over 4,000 inmates of the State Institution for the Insane in order to obtain results on a group of people likely to remain located indefinitely, whereas the children in institutions often make but a short stay and the majority will have left in a few years.

Table I gives the test results in 28 children that have remained in one institution for five years.

It is seen that when once antitoxin develops in those injected with toxin-antitoxin it is quite or almost as persistent as in those who develop it naturally. It lasts in the great majority for at least five years.

Response to Toxin-Antitoxin Stimulation in Infants. Over 2,400 infants ranging in age from a few hours to two weeks were tested and immunized by Miss Denny, Miss Alper and the writer. When re-tested one year later we found that only 50 per cent. had become immune. This showed that active immunity is not readily produced in these young infants by toxin-antitoxin injections owing partly to the inhibitive effect of the passive immunity derived from the mother. This passive immunity is lost later. It is found that toxin-antitoxin can only be depended upon to act effectually after the age of six months.

TABLE I.

SHOWING RESULTS OF 28 CASES DURING 5½ YEARS RE-TEST PERIOD

	Schick Test	Toxin-Antitoxin—3 injections			Schick Re-tests during 5 years							
	Nov. 16, 1915	Nov. 20 & 25,		Dec. 3, 1915	March 20, 1916	May 19, 1916	June 29, 1916	Nov. 9, 1916	July 22, 1917	Jan. 13, 1919	Jan. 11, 1921	Jan. 1922
Case 1	++	+	+	+	—	—	—	—	—	—	—	—
2	+	+	+	+	—	—	—	—	—	—	—	—
3	+	+	+		—	—	—	—	—	—	—	—
4	±	+	+		±	±	—	—	+ =	—	—	—
5	+	+	+	+	—	—	—	—	—	—	—	—
6	++	+	+	+	+ =	—	—	—	—	—	—	—
7	±	+	+	+	—	—	—	—	—	—	—	—
8	±	+	+		—	—	—	—	—	—	—	—
9	++	+	+	+	—	—	—	—	—	—	—	—
10	+	+	+	+	—	—				—	—	
11	±	+	+		—	—	—	—	—	—	—	These children were lost track of.
12	+	+	+	+	—	—	—	—	—	—	—	
13	+	+	+	+	—	—	+ =	—	—	+	—	
14	++	+	+	+	—	—	—	—		—	—	
15	+	+	+	+	—	—	—	—	—	—	—	
16	+	+	+	+	—	+ =	—	—	—	—	—	
17	+	+	+	+	—	—	—	—	—	—	—	
18	+	+	+	+	±	—	—	—	—	—	—	
19	+	+	+	+	+ =	—	—	—	—	—	—	
20	±	+	+		+ =	±	—	+ =	+ =	±	—	
21	+	+	+	+	±	—	—	—	—	—	—	
22	±	+	+	+	—	—	—	—	—	—	—	
23	+	+	+	+	—	—	—	—	—	—	—	
24	+	+	+	+	—	—	—	—	—	—	—	
25	++	+	+	+	—	—	—	—	—	—	—	
26	±	+	+		—	—	—	—	—	—	—	
27	+	+	+	+	+	—	—	+	+ =	—	—	
28	+	+	+	+	—	—	—	—	—	—	—	
	*Thirteen received 2 injections 1½ c.c. 1-27-19. *Twenty received 2 injections 1½ c.c. 1-27-19. — = negative. + = positive.				4± 3+ = 21—	2± 1+ = 25—	1+ = 27—	1+ 1+ = 26—	3+ = 25—	2+ 26—	28—	

Twenty-one of 28 became negative within 4 months and remained negative 5 years.
Twenty-five of 28 became negative within 6 months and remained negative 4½ years.
The remaining 3 became — but relapsed more or less; but became permanently — after two additional injections.
Only nine remained to 1922. These were all negative.

TABLE II.

	Total Tested	Doses T A T	Months After	Shick Results		Re-test Percentage Becoming Immune
				11—		
St. Joseph's	12	3	60	*1+		100
Ages mostly	38	2 or 3	*38	38—		100
				Boys	Girls	
				64—		
5—10 years	78		10	9+	5+	94
				*91—		
	120	2 or 3	3	11+	18+	85
	8	1 or 2				
St. Agnes	3	2	60	3—		100
3—14 years	25	2	20	25—		100
				Girls	Boys	
	54	2	12	50—	4+	92
	38	2 or 3	38	33—	2+	94.8
St. Dominick				6—	1—	
Ages mostly	10	1	31	1+	2+	70
9—12 years				14—		
	16	2	18	1+	1+	93.8
St. Malachy's				13—		
Ages (5—15 yrs.)	14	2	14	1—		100
Randall's Island				42—	4—	89.2
(5—30 years)	49	2	33	2+	1+	94.9
Hebrew Orphan	68	3	32	59—	3—	92.6
Asylum (5—15 yrs.)				4+	2+	
Mt. Loretto	83	1 or 2	3	62—	12—	
				3+	7+	77.5
House of Refuge	7	1 or 3	35	4—	2—	71.5
(10—20 yrs.)				1+		
Leake & Watts	32	3	27	26—	2—	93.8
(5—15 yrs.)				3+		
					1+	
	23	2 or 3	24	19—	3+	82.6
N. Y. Catholic	19	3	6	17—	2+	89
Protectory				1+		
(4—10 yrs.)	20	3	12	18—	1+	95
	55		22	53—	2+	96.4
N. Y. Foundling	32	3	17	30—		96.9
(1—5 yrs.)				1+	1+	
Chldrns. Stations	350	3	7	314—	37+	89
3 mos. to 4 yrs.						

*1 case became positive, but when reinjected became negative.

The results upon re-test were as follows:

Group	No. of Cases	Reaction Changed From Positive to Negative	Reaction Remaining Positive	Percentage Becoming Immune
A	105	85	20	80%
B	245	209	36	85%

Response in School Children. Another side of the problem was presented by the use of toxin-antitoxin among the public school population. During the past two years fully 50,000 children attending the public schools of Brooklyn have been Schick tested and immunized under my personal supervision.

During the past month we have begun re-testing those children who were immunized.

The results are given in Table III:

School	No. of Children Retested	No. of Children Becoming Negative	No. of Children Remaining Positive	Percentage Becoming Immune
P.S. 16, Bklyn.	288	260	28	90%
P.S. 142, Bklyn.	157	148	9	94%
P.S. 50, Bklyn.	125	119	6	95%

The above children received three doses of 1 c.c. each. In P. S. 149, Brooklyn, 35 children received two doses of 1½ c.c. each. Of these children, 26 became negative, 9 remained positive and were re-injected. The latter will be tested again later.

Acquired Immunity in Adults. During the past year 4,396 inmates of Kings Park State Hospital were Schick tested and 555 patients were immunized. These patients were divided into two groups, Group A receiving one inoculation of toxin-antitoxin and Group B, two inoculations in 1½ c.c. doses.

Such, in brief, is a résumé of part of the work done during the past 5 years to test out the possibilities of the duration of conferred immunity by means of diphtheria toxin and antitoxin mixtures.

For bibliography see the original article.

ACCURACY OF THE SCHICK REACTION

Influence of Variations in Diphtheria Toxin Content in Schick Outfits

Abraham Zingher

The rapid increase in the popularity of the Schick reaction as a method for determining the susceptibility or immunity of individuals to diphtheria is based on two factors: (1) the great reliability of the test, and (2) the ease with which it can be carried out.

Dr. Park and I have repeatedly emphasized the need of observing with care three points that are absolutely essential if the test is to be reliable. They are: (*a*) a standard toxin dilution of proper strength; (*b*) accurate technic in the injection by means of a good syringe and a suitable fine needle, and (*c*) an accurate interpretation of the reaction.

Reliability of the Schick Test

Accumulating experience and reports from many sources, and our own experience at the Willard Parker Hospital and in various other institutions during a period of seven years, have established the Schick reaction as a most valuable clinical test for determining immunity from diphtheria or susceptibility to the disease. Most striking, indeed, have been the results noted at the Willard Parker Hospital. Of several thousand scarlet fever patients, more than 2,200 had given a negative Schick reaction on admission to the institution. No prophylactic injections of antitoxin were given to these patients, as they were considered to be immune to diphtheria. They were carefully observed and, it is interesting to note, not one of these patients developed undoubted clinical diphtheria even though they were subsequently exposed to cases of diphtheria developing among some of the Schick-positive patients; indeed, many of them had become temporary carriers of virulent diphtheria bacilli during their stay in the hospital.

The great reliability of the test is further emphasized by our recent experience in several institutions in which outbreaks of diphtheria had occurred. The Schick test was made on all the children of the institutions, and only the positive reactors were passively immunized with antitoxin. In each institution the outbreak was thus promptly controlled, and no cases of diphtheria developed among the Schick-negative children, although they had received no immunization with antitoxin.

It is important from a practical standpoint to remember that individuals who are carriers of virulent or nonvirulent diphtheria bacilli may develop a septic sore throat due to the streptococcus or some other organism. Such carriers as a rule give negative Schick reactions and are therefore immune to diphtheria. The presence of a lesion resembling a diphtheritic exudate in these individuals, associated with a positive throat culture, would be sufficient evidence to many observers that the lesion was diphtheria. In several such patients, however, in whom we were certain that the negative Schick reaction was correct, antitoxin was not injected, although we were prepared to give it if the symptoms became worse. Repetition of the Schick test again gave negative results. Specimens of blood were taken from these patients and their antitoxin content determined. All these patients had from 1/10 to 2 units of antitoxin per cubic centimeter of blood serum which, according to von Behring, would be from ten to 200 times as much as was necessary to protect the individuals from diphtheria. Antitoxin, however, should always be given when the suspicion of the presence of diphtheria is strong, because there is always the possibility that there has been an error in the technic of the test or in its reading, and we are not justified in taking unnecessary risks. Failure of improvement in such a case after the administration of antitoxin will generally show that we are dealing with a nondiphtheritic sore throat in a carrier of diphtheria bacilli.

Sources of Possible Error Connected With the Schick Test

I have been informed by various observers of cases in which patients giving negative Schick reactions subsequently developed diphtheria. I firmly believe that in such cases one or more of the three factors enumerated above, i. e., the strength of the toxin, the technic of the injection, or the interpretation of the reaction, had been at fault. In several instances we were able to show that the technic itself was inaccurate. Errors in the reading or the interpretation of the test accounted for some of the poor results. At times a Schick reaction was read as negative after a superficial observation at the end of twenty-four or forty-eight hours, when a later reading would have disclosed a positive reaction. This was indicated by the definite pigmentation remaining at the site of the test and seen at a subsequent examination. Such pigmentation should have been looked for and would probably have been found in some of the cases that were reported as clinical diphtheria developing in Schick-negative children. To interpret and identify the pseudoreaction, moreover, it is necessary to make a control test with the heated toxin as I have recommended. When a large number of very young children are to be tested, the control could often be omitted for the sake of economy in time, in view of the relative infrequency of the pseudoreaction in this age group.

The first essential condition for a reliable Schick test is to have a standard toxin dilution of proper strength. It is possible for the individual observer to guard against faulty technic and inaccurate reading of the reaction, but he is forced to depend on the outfits supplied by the various laboratories for a suitable toxin preparation. It is important to realize that individuals who give a strongly positive Schick reaction with standard dilutions of toxin may give only a faintly positive or *a negative* reaction with weaker dilutions.

Table 1 illustrates the effect of three different dilutions of toxin on groups of children who had given strongly positive, moderately positive and faintly positive reactions, respectively, with a standard dilution. Variations

TABLE 1.
EFFECT OF THREE DILUTIONS OF TOXIN*

	Groups of Children		
Dilutions of Diphtheria Toxin	1	2	3
1. Standard dilution, 0.2 c.c. = 1/50 M. L. D.....	+	±	$\underline{\pm}$
2. One-half standard dilution, 0.2 c.c. = 1/100 M. L. D.	±	$\underline{\pm}$ or —	—
3. One-quarter standard dilution, 0.2 c.c. = 1/200 M. L. D.	$\underline{\pm}$ or —	—	—

*The signs used in this and the following tables should be read thus: + strongly positive Schick reaction; ±, moderately positive Schick reaction; $\underline{\pm}$, faintly positive Schick reaction; —, negative Schick reaction.

in the reactions shown by the same individuals to apparently the same dilutions of toxin can thus be explained by differences in the strength of the dilutions used in the test.

My attention was recently drawn to the possibility that some of the outfits supplied by commercial laboratories were below standard strength. A private physician reported that he had used a Schick outfit from a commercial laboratory on three children in one family and had obtained negative reactions in all the children. He suspected that something was wrong with the toxin dilution, and retested the three children with an outfit from the Research Laboratory. Two of the children now gave positive reactions and one a negative reaction. The importance of this observation prompted me to ask several commercial laboratories for a number of Schick outfits, which they manufacture for public sale, and to test them. These outfits were secured directly from the manufacturers so as to avoid the possibility that the outfits had deteriorated while in the hands of the retailer. A series of children who had given positive Schick reactions with the outfits furnished by the Research Laboratory was used for the tests. All the outfits from one laboratory were tested simultaneously on the same children, and at the same time a control test was made with one of the outfits of the Research Laboratory.

The results noted were very interesting and are of considerable importance in showing one of the great possible sources of error in the results of the Schick test.

Results Obtained with Commercial Laboratory Outfits

In reading Table 2 and the subsequent tables the vertical columns indicate the different reactions given by the various outfits on the same child, and the horizontal columns the reactions shown by one outfit on various children.

Table 2 shows that Outfits 1 and 2 were inactive and gave negative reactions in all children. It is important to note that not even a faintly positive reaction was noted in those children who had shown a strongly positive reaction with the control test. Outfit 3 gave reactions about twice as strong as those noted with the control test, while Outfits 4 and 5 gave reactions similar to those shown with the control test.

TABLE 2.

RESULTS WITH OUTFITS OF COMMERCIAL LABORATORY 1.

	Children Tested						
Schick Outfits	A	B	C	D	E	F	G
Control (Research Lab.)	+	+	+	±	$\underline{\pm}$	±	+
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	++	+	++	+	±	+	++
4	+	+	+	±	$\underline{\underline{\pm}}$	+	+
5	+	+	Not tested	+	$\underline{\pm}$	+	Not tested

Table 3 shows that Outfit 1 gave a moderately positive reaction in one child showing a strongly positive reaction with the control test, and faintly positive reactions in two children giving moderately positive reactions with the control outfit.

TABLE 3.

RESULTS WITH OUTFITS OF COMMERCIAL LABORATORY 2

Schick Outfits	Children Tested A	 B	 C
Control (Research Lab.)	+	±	±
1	±	$\underline{\pm}$	$\underline{\pm}$
2	$\underline{\pm}$	$\underline{\pm}$	$\underline{\pm}$
3	$\underline{\pm}$	—	—

Outfit 2 gave a faintly positive reaction in the child showing a strongly positive reaction with the control test, and very faintly positive reactions in the two children giving moderately positive reactions with the control outfit.

Outfit 3 gave a very faintly positive reaction in the child showing a strongly positive reaction with the control test, and negative reactions in the two children giving moderately positive reactions with the control test.

The directions for the tests given in the circulars of the outfits of Laboratory 3 state that 0.1 c.c. should be injected. We injected 0.2 c.c. so as to make the area of the reaction conform to that of the control test, in which 0.2 c.c. is used. In Child E (Table 4), Outfits 1 and 2 gave negative reactions when 0.1 c.c. was injected, but faintly positive reactions when 0.2 c.c. was injected.

TABLE 4.

RESULTS WITH OUTFITS OF COMMERCIAL LABORATORY 3

Schick Outfits	Children Tested A	 B	 C	 D	 E
Control (Research Lab.)	+	+	+	+	±
1	+	+	+	+	$\underline{\pm}$
2	+	+	+	+	$\underline{\pm}$
3	+	+	+	+	±
4	±	±	±	+	$\underline{\pm}$

Table 4 shows that three of the outfits gave reactions similar to the control test in four children; the fifth child showed a faintly positive reaction with Outfits 1 and 2. Outfit 4 gave moderately positive reactions in three of the children who had strongly positive reaction with the control outfit. The capillaries containing the toxin in these outfits were of fairly thick glass, and one experienced considerable difficulty in breaking them to make the necessary dilution. The clinical results with the outfits of this laboratory corresponded quite closely to those noted with the control outfit. It must be remembered, however, that we used twice the amount of diluted toxin directed by the circular in these outfits, and injected 0.2 c.c. instead of 0.1 c.c. in making the tests.

The contents of one capillary were also injected into a guinea-pig weighing 250 gm. Each capillary was supposed to contain one minimal lethal dose of toxin; therefore the animal should have died on the fourth day after receiving the injection. The guinea-pig showed local induration, lost in weight and became quite ill, but ultimately recovered. This result showed that the capillary contained less than one minimal lethal dose of toxin, the amount required to make the standard dilution for that outfit.

Table 5 shows that the outfits of this laboratory gave results which corresponded quite closely to those noted with the control outfit. Outfit 5 was perhaps slightly stronger than the control.

TABLE 5

RESULTS WITH OUTFITS OF COMMERCIAL LABORATORY 4

Schick Outfits	Children Tested			
	A	B	C	D
Control (Research Lab.)	+	±	±	+
1	+	±	+	+
2	+	±	±	+
3	+	±	±	+
4	+	±	±	+
5	+	±	+	+

Results Obtained with Research Laboratory Outfits

In order to determine how uniform the outfits of the Research Laboratory are, several of them were tested in the same manner as the outfits supplied by the commercial laboratories.

This outfit is prepared at the Research Laboratory with a stable ripe toxin, of which the minimal lethal dose is 1/70 c.c. The capillary tubes of the outfits are filled by means of a 0.25 c.c. original record syringe (the Kny-Scheerer Company) the barrel of which is divided into fifteen equal parts. One subdivision of the syringe corresponds to 1/60 c.c., and this is the amount of toxin filled into each capillary tube. The contents of the capillary tube represent, therefore, a little more than one minimal lethal dose of toxin, the slight excess (about 14 per cent.) allowing for some deterioration of the toxin. When the toxin is diluted with the 10 c.c. of saline contained in the outfit, the solution contains 1/50 minimal lethal dose in 0.2 c.c., the amount recommended for the test. Allowing for wastage of some of the diluted toxin, one outfit will be sufficient for at least thirty-five Schick tests.

Table 6 shows that the first four outfits gave quite uniform results. Outfit 5 gave a moderately positive reaction in Children A and C, who had shown strongly positive reactions with the other outfits. A slightly deeper injection may account for these reactions.

TABLE 6.

RESULTS WITH RESEARCH LABORATORY OUTFITS

Research Lab. Schick Outfits	Children Tested				
	A	B	C	D	E
1	+	+	+	±	⩲
2	+	+	+	±	⩲
3	+	+	+	±	⩲
4	+	+	+	±	⩲
5	±	+	±	±	⩲

Three guinea-pigs, weighing 250 gm., were each injected with the contents of one of the Research Laboratory outfits One animal died of acute diphtheria intoxication between the third and fourth day, and the other two died between the fourth and fifth day.

The results noted with these outfits show a considerable degree of uniformity in the clinical tests. The animal tests also show that each of the outfits contained one unit of toxin, the amount required for a proper standard dilution.

It is evident, therefore, that it is possible to manufacture outfits for the Schick test on a large scale without sacrificing accuracy.

Summary and Conclusions

1. The accuracy of the Schick test depends on the strength of the toxin, the technic of the test and the interpretation of the reaction.
2. Errors in any of these may make the results of the test unreliable. It is possible to guard against a poor technic and a wrong interpretation of the reaction, but it is essential to be able to depend on the accuracy of the outfits for the Schick test obtainable on the market.
3. The outfits furnished by four commercial laboratories were tested. Of five outfits of one laboratory, two contained too small an amount of toxin to give a positive reaction in Schick-positive children. Of three outfits of the second laboratory, one was somewhat weaker and two very much weaker than the standard outfit, so that negative reactions were obtained in Schick-positive individuals. The outfits of the third laboratory compared fairly well with the control in the clinical test when twice the amount of toxin dilution prescribed in the directions on the outfits was injected. The animal test showed only about 85 or 90 per cent. of a minimal lethal dose in an outfit instead of one minimal lethal dose. The outfits of the fourth laboratory gave reactions similar to those noted with the control test.
4. The outfits furnished by the Research Laboratory gave practically uniform results clinically, and the animal test showed full potency of the toxin.
5. It is practicable to manufacture outfits for the Schick test on a large scale without sacrificing accuracy.

DIPHTHERIA PREVENTIVE WORK IN THE PUBLIC SCHOOLS OF NEW YORK CITY

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By Abraham Zingher

1. *Introduction.* The very successful results obtained by us and by other workers in the active immunization of large numbers of children with diphtheria toxin-antitoxin has stimulated the Department of Health of New York City to begin an active campaign in diphtheria preventive work in the public and parochial schools of this city. Permission was obtained from the Department of Education to carry out the Schick test and the necessary active immunization in about 250 public schools. Beginning in 1916, a number of schools were Schick tested in the Boroughs of Manhattan, the Bronx and Brooklyn. During the period of the war the work was limited in extent on account of lack of necessary personnel.

Through the coöperation of the Manhattan Chapter of the American Red Cross, Dr. Park obtained the financial aid necessary to start this work on a more extensive scale. A number of physicians, nurses and assistants were employed and divided into groups. In Brooklyn and Queens the work was done under the direction of Dr. Schroeder while in Manhattan and the Bronx, 2 groups have been working under direction of the writer. Between the end of February, 1921, and the end of this school year, we have applied the Schick test in 44 of the larger schools in these 2 boroughs and have tested over 52,000 children in Manhattan and the Bronx. Those children who gave a positive or a positive combined reaction were injected with toxin-antitoxin. In the first 22 schools, the injected children were re-tested with the Schick test before the end of the school year, in order to determine which children had developed an active immunity against diphtheria. The following was the method followed in the schools.

2. *Introductory Procedure Before Schick Testing a School.* An interview was first obtained with the principal or assistant principal, the Schick test and toxin-antitoxin immunization were explained, and the coöperation of the school authorities was invited. Consent blanks and circulars of information to parents were left at the school to be distributed to all the children. Conference meetings were arranged at which the teachers were addressed by the principal or a physician, and their coöperation also requested. Each child was required to return the consent blank signed by the parent, with an affirmative or negative statement. The teachers later made out alphabetical lists of the names of those children whose parents had given their consent. Special sheets, called class sheets, were used for this purpose and also for recording subsequently the results of the Schick test and the injections of toxin-antitoxin.

Procedure on the Day of the Schick Test. On the day of the test, the children are brought to a class room where the physicians and nurses are doing the work. By good coöperation on the part of the principal and teachers, we are able to apply the Schick Test and the control test to as many as 500 or 600 children per hour. In one school we tested on one day, during school hours, 2400 children; in another double school, over 2700 children. The needles are thoroughly cleaned and sterilized between the tests by wiping them off with a cotton sponge which has been placed in a Petri dish and saturated with 95 per cent. ethyl alcohol. A 1.0 c.c. "Original Record" syringe and a 26 gauge ¼ inch steel needle were found very serviceable for the test.

4. *The Dilutions for the Schick Test and for the Control Test.* The Schick outfits[1], so generally used at the present time and so well adapted to the work where small numbers are to be tested, are not very suitable for the testing of large numbers of children. The dilutions for our work are therefore prepared from bulk toxin, as described in a previous communication[2]. The dilutions are made according to the M.L.D. strength of the toxin. Our toxin had an M.L.D. of 0.01 c.c. A dilution of 1.0 c.c. of toxin in 1,000 c.c. of saline gives a strength of toxin of which 0.2 c.c.=1/50 M.L.D. To make the reactions more distinct, we add 25 per cent. excess of toxin and dilute 1.25 c.c. of toxin in 1,000 c.c. of saline. The dilution thus prepared represents 1/40 M.L.D. in each 0.2 c.c.

For the control test dilution. Instead of 1.25 c.c., we use 1.5 c.c. of toxin, heated to 75°C. for ten minutes and diluted with 1,000 c.c. of saline. The 20 per cent. excess of heated toxin in the control dilution as compared with the unheated toxin used in the Schick dilution was added for the purpose of allowing for any deterioration that might have taken place in the bacillus protein during the process of heating. By observing this precaution we found that children showing a negative pseudo-reaction would have an area of redness and later pigmentation equal in size and appearance on both forearms. In consequence there was less danger of mistaking a negative pseudo-reaction for a combined positive reaction.

The dilutions were freshly prepared each day for the day's work. No diluted toxin was kept over from one day to another.

The rule was adopted always to make the Schick test on the right forearm about 2 to 3 inches below the bend of the elbow and the control test on the left forearm in a corresponding location. One knew then exactly where to look for the reactions and could thus avoid confusion in the readings.

Procedure on the Day of the Readings of the Schick Reaction and of the First Injection of Toxin-Antitoxin. The reactions were read from 3 to 7 days after the Schick tests. By reading the tests before three days have elapsed the interpretation of the reactions is not as accurate. Readings made on the third and fourth days showed the fading pseudo-reactions somewhat better, but a few doubtful reactions still remained, especially of the positive combined type. These were more accurately interpreted at the later readings. Very faintly positive Schick reactions, however, were likely to show no redness and only a slight brownish pigmentation when they were seen on the seventh day.

I. *Results of Schick Test.* The results with the Schick test in 44 schools are interesting and significant. They show that:

1. Children from the homes of the more well to do have a much higher percentage of positive Schick reactions than those from the homes of the poorer classes of the population, who live in closely crowded neighborhoods. The table shows that in some schools as many as 67.0 per cent. of the children were found to give the positive reaction. The percentage diminishes as we follow the schools down on the table until we reach schools located in the densely congested sections of the East Side, where not more than 16 to 20 per cent, gave positive reactions.

2. *Contact immunity* would seem to be an important element in the establishment of the so-called "natural immunity." Repeated exposure to the diphtheria bacillus in the congested districts not only causes actual clinical cases of diphtheria to develop but also probably produces mild infections

of the mucous membranes which are not recognized as diphtheria, but which may lead to the gradual development of an antitoxic immunity. This theory has received striking support from the results of the Schick testing of two private schools and one rural school*. At the Lawrenceville School near Trenton, N. J., 79 per cent. of boys, varying in age from 12 to 21 years, gave positive reactions; at the George School, Georgetown, Pa., 75 per cent. of the children gave positive reactions. In a rural school at Shilo, Cumberland Co., N. J., Dr. Knight of the New Jersey State Department of Health found that 85 per cent. of the children gave positive Schick reactions. These are striking figures and indicate that segregation of the children, either among the well to do or in rural and sparsely settled sections, probably plays an important part in retarding the development of natural immunity to diphtheria.

3. A study of the racial types, as represented by the child population of the different schools, gives other interesting data and shows that the *racial factor* must also be considered as one of the influences in the development of natural immunity.

(a) Children of the colored race, living often in congested neighborhoods, showed in spite of the crowded conditions a high proportion of positive Schick reactions. I had had occasion to note this racial peculiarity seven years ago at the Howard Colored Orphan Asylum at Kings Park, L. I., and a year later at the Colored Orphan Asylum at Riverdale-on-the-Hudson.

(b) Children of Italian extraction, living in the crowded East Harlem section of New York City, gave the lowest percentage of positive reactions.

(c) Children of Bohemian and Irish extraction gave about one-third positive Schick reactions.

(d) Children of Hebrew parents varied considerably in the percentage of positive Schick reactions. Those living in the congested sections of the City showed a low percentage, while those living in the upper West Side section of the City were among those who gave the highest percentage of positive reactions.

4. The *family factor* is also of importance in the development of natural immunity. This was brought out in some of our earliest observations on the results of the Schick test in groups of children belonging to the same family. We found that there was a marked tendency for all the children of the same family to show a similar Schick reaction, whether it was positive or negative. Where variations were found in the reaction, the younger children as a rule gave the positive, the older chlidren, the negative Schick reactions. The reverse condition, where older children gave a positive and younger children a negative reaction was very rare, except in families with young infants, who often gave a temporary negative reaction due to maternal immunity. These observations have been repeatedly confirmed in our more recent work in the public schools. While as a rule the living conditions and consequent exposure to diphtheria infection are very similar for all the children of the same family, yet we have seen that different families living under closely parallel conditions often show entirely different Schick reactions. With regard to antitoxin production there is probably an hereditary tendency on the part of the children of one family to respond either readily or slowly and poorly to repeated mild infections with the diphtheria bacillus.

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*I am indebted to Mr. Bowen, Secretary of the New Jersey State Department of Health for the results of the Schick test at the Lawrenceville School, and to Dr. David F. Weeks, Superintendent of the State Hospital for Epileptics at Skillman, N. J., for the results at George School.

This organism is as a rule universally prevalent in larger communities. For example, in New York City we always have on an average about 4 to 5 per cent. of bacillus carriers.

5. Natural antitoxic immunity in human beings can be interpreted therefore as due to a combination of factors in which *contact immunization* seems to play the most important part. The *racial* and *hereditary family factors* however, must also be considered as important elements in the development of such natural immunity.

6. The results show a fairly high proportion of negative pseudo-reactions among children of school age. It is interesting to note that there is a much higher proportion of negative pseudo-reactors among the immunes than there is of positive combined reactors among the non-immunes. We can therefore assume that repeated exposure to infection with the diphtheria bacillus brings about not only an antitoxic immunity, but also a hypersensitiveness of certain predisposed individuals to the diphtheria bacillus protein. The development of this hypersensitiveness renders the cells of the epidermis of the pseudo-reactor anaphylactic to the autolyzed protein of the diphtheria bacillus which is present in the test solution of toxin. The infrequent exposure of positive reactors to infection with the diphtheria bacillus accounts for the smaller number of positive combined reactions among them.

Among the colored children we noted a relatively low percentage of pseudo-reactions. There seems to be a racial tendency in these children to respond poorly to the antigenic action not only of the diphtheria toxin, but also of the bacillus protein.

II. *Results with Toxin-Antitoxin Immunization.* Table 1 shows results in a few of the schools in which the children receiving the toxin-antitoxin injections were re-tested. P. S. 90 was the only school in which the children had received *three* injections of toxin-antitoxin and the re-test was made after *five months.* In this school 87.5 per cent. of the injected children were found to have become immune as shown by a negative Schick reaction. The other injected children gave reactions that were still positive, but were very much fainter than the original reactions.

In the other re-tested schools, only two injections of toxin-antitoxin had been given from two to two and a half months previously. Two of these schools (P. S. 101 and 57), located in the more densely crowded sections of the City, showed 76.1 and 64.5 per cent. positive Schick reactions respectively. These results compare favorably with those noted in the other schools given in the table, in three of which we had used the same preparation of toxin-antitoxin (P. S. 43 Manhattan; 186 and 132). In the last two schools, however, the Schick re-tests were made after two months. In the remaining two schools noted in the table (P. S. 43 Bronx, and 46), a different mixture of toxin-antitoxin was used, but the results were about the same.

One feature of the Schick re-tests was noticeable. Most of the children who continued to give the positive reaction showed a much fainter area of redness than in the original test. In many instances the brownish pigmentation of the original positive reaction was still visible and was even at this time much larger in diameter than the area of redness of the re-test. The fainter reactions in the re-tests stood out also in striking contrast to the strongly positive reactions of children who have received the Schick tests for the first time.

Children who showed a positive reaction, even though it was very small in size and faint in appearance, received one or two additional injections of toxin-antitoxin. Schick re-tests will be made again next fall and we expect

TABLE 1

RESULTS OF RE-TESTS AFTER DIPHTHERIA TOXIN-ANTITOXIN IMMUNIZATION IN NEW YORK CITY PUBLIC SCHOOLS

P.S.	Location	Time Since Injection	Preparation Used	Doses Given	Amount	Total Children Retested	Schick Pos.	Schick Neg.	Per Cent. Neg.
90	148th St. & 8th Avenue	5 mo.	No. 24	3	1 c.c.	160	20	148	87.5
101	111th St. & Lexington Avenue	2.5 mo.	No. 23	2	1.5c.c.	152	31	121	76.1
57	115th St. & 3rd Avenue	2.5 mo.	No. 23	2	1.5c.c.	254	90	164	64.5
168	105th St. & 2nd Avenue	2 mo.	No. 35	2	1.5c.c.	213	98	115	52.7
43 Man.	129th St. & Amsterdam Avenue	2.5 mo.	No. 23	2	1.5c.c.	183	103	80	43.8
43 Bx.	135th St. & Brown Place	2.5 mo.	No. 35	2	1.5c.c.	397	257	140	35.2
186	145th St. & Broadway	2.0 mo.	No. 23	2	1.5c.c.	291	206	85	29.9
132	182nd St. & Wadsworth Avenue	2.0 mo.	No. 23	2	1.5c.c.	213	154	59	27.7
46	St. Nicholas Avenue & 157th Street	2.0 mo.	No. 35	2	1.5c.c.	302	226	76	25.1
157	127th Street and St. Nicholas Avenue	2.0 mo.	No. 35	2	1.5c.c.	253	199	54	21.3

from the results of our previous experience that most of these children will then given a negative reaction.

III. *Age incidence in Relation to Active Immunization Against Diphtheria.* The child population can be divided for purposes of active immunization into five distinct groups.

TABLE 2

Importance of Age in Active Immunization With Toxin-Antitoxin.

A. *Infants Under Six Months.*	
(a) Under three months	Children mostly immune (maternal). Do not develop an active immunity after toxin-antitoxin injections.
(b) Three to six months	Generally found to be immune (maternal). Toxin-antitoxin may be used, but it is not as effective as when used in the following age-group. A negative Schick test cannot be depended upon to indicate a permanent immunity.
B. *Pre-School Age.*	
(a) Six months to two years	Schick test can be omitted in this group, as the proportion of positive reactors is very high. A negative Schick reaction cannot be depended upon to indicate a permanent immunity. Strongly advisable to give toxin-antitoxin *to all these children.*
(b) Two to five years	Schick test may be used first. Positive reactions very high. Procedure can be greatly simplified by omitting Schick test and injecting *all these children with toxin-antitoxin.*
C. *Public School Age.*	
(a) Five to six years	Incoming classes (Kindergarten and 1A). Schick test may be used first. Positive reactions over 65 per cent. *Strongly advisable to inject all these children with toxin-antitoxin.*
(b) Six to fifteen years	Schick test and control test *should* be used *first.* Many negative pseudo-recations must be excluded. Toxin-antitoxin for Schick positive and positive combined reactors only.
D. *High School Age.*	
(a) Fifteen to nineteen years	Schick test and control test should be used *first.* Many negative pseudo-reactions must be excluded. Toxin-antitoxin for Schick positive and positive combined recators only.
E. *Adolescents and Adults.*	
(a) Nineteen years up	Schick test and control test should be used *first.* Many negative pseudo-reactions must be excluded. Toxin-antitoxin for Schick positive and positive combined reactors only.

Table 2 gives in a condensed form the indications for the Schick test and toxin-antitoxin immunization.

A. Infants under six months are protected temporarily in fully 85 to 90 per cent. by an inherited maternal antitoxic immunity against diphtheria. The inherited antitoxin not only protects these children, but it is also interferes with the development of an active immunity, when they are injected with toxin-antitoxin.[5] It is, therefore, important to wait until most of this inherited antitoxin has been eliminated before attempting to inject the children with the toxin-antitoxin.

B. Probably the most important period of life in which the toxin-antitoxin should be used is from *six months to two years.*[6] A negative Schick reaction in this group cannot be entirely depended upon, as the reaction later changes in many of these infants from negative to positive (loss of maternal immunity). It is therefore *strongly advisable* to omit the Schick test and inject *all children* in this group with the three doses of toxin-antitoxin.

To make certain that we reach all of the susceptible children, it is just as strongly advisable to inject also *all children from two to five years of age.* The negative Schick reaction in this age group is probably a permanent index of natural immunity. The proportion of susceptible children, however, is very high and the *omission* of the Schick test would simplify this important immunizing procedure for many physicians and thereby make it more certain that a greater number of them would recommend and use it. The simple subcutaneous injection of three doses of toxin-antitoxin would appeal to many physicians who are not thoroughly acquainted with the technique or the interpretation of the Schick test.

The high proportion of positive Schick reactions in children from six months to five years of age and the corresponding high morbidity and mortality from diphtheria in this group (80-85 per cent. of all diphtheria cases) are strong indications for the active immunization of *all children under five years of age.* The injections of toxin-antitoxin produce very little pain and discomfort in these young children because only very few are hyper-sensitive to the bacillus protein as shown by the small percentage of negative pseudoreactions among them. The statement may even be definitely made that the younger the child the more tolerant it is to the injections of toxin-antitoxin.

At all ages it should be remembered that no individual can be pronounced immune to diphtheria until he has been proven to be so by the Schick test. Until this is done it is always a question in each case whether the particular child has responded to the toxin-antitoxin stimulation. For this reason the Schick test must never be omitted as a final part of the procedure of active immunization against diphtheria.

The facts indicate that the solution of the diphtheria problem depends upon just such a general active immunization of all children of pre-school age. Compulsory immunization would be a great forward step in this direction, if public opinion could be converted by educational propaganda to the advisability of such a step.

C. The children of school age can be divided into two groups: (a) the entering classes and (b) the higher grades.

(a) The entering classes comprise in the educational system of New York City the Kindergarten and 1-A Grades. This group is of special interest to the health official, as it is through these classes that he is enabled for the first time to exercise his influence more directly upon the young children of the community. For the reasons stated above and until we shall have established the principle of actively immunizing *all* children of pre-school age against diphtheria, it will be advisable to inject *all children of the incoming classes* in our public schools with toxin-antitoxin. The omission of the Schick test will simplify the work for the school physician or health officer, who will then be more apt to recommend and use the toxin-antitoxin. If this procedure is followed, there will be created within a few years a diphtheria immune school population. Until we can do this as a routine we shall have to take into account for the present at least the many susceptible children who are now in the higher grades.

(b) In the second group, Grade 1-B and upward, the Schick test and control test should be used before immunization for the following reasons: 1. In many schools the number of positive Schick reactions may not be more than 16 to 25 per cent. In such schools by a preliminary Schick test many children can be saved from getting unnecessary injections of toxin-antitoxin. 2. From 10 to 25 per cent. of children of school age give a negative pseudo-reaction. These children are not only immune to diphtheria, but they are the very ones who would have *severe local and constitutional reactions* after injections of toxin-antitoxin.

School children who give a positive or positive combined reaction should receive the three injections of toxin-antitoxin. Children who have a positive combined reaction will generally show moderate or fairly severe local and constitutional symptoms, but most of these symptoms subside after two or three days. Over 50,000 injections of toxin-antitoxin, which we have given during the past four months, we have not seen a single serious result or a single infection. This statement must be emphasized as the swelling of the arm at the site of injection leads some of the parents to think that the child has "blood poisoning."

D. and E. Children over fifteen years of age and adults should always have the Schick test and control test applied before immunization to determine their susceptibility to diphtheria. It is in these individuals that we find the more marked forms of negative pseudo-reaction which should be identified as such and not mistaken for positive or positive combined reactions. It may be left optional with the adult individual who has a positive combined reaction whether or not he should receive the toxin-antitoxin.

For bibliography see the original article.

THE SCHICK TEST PERFORMED ON MORE THAN 150,000 CHILDREN IN PUBLIC AND PAROCHIAL SCHOOLS IN NEW YORK (MANHATTAN AND THE BRONX).

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Abraham Zingher

Performance of the Schick test and active immunization with toxin-antitoxin were started on a large scale in the public schools of New York during the spring of 1921. Since then the work has been continued under the general direction of Dr. Park, and nearly all the children in public and parochial schools in Manhattan and the Bronx have been tested by myself and my group of assistants. In Brooklyn and Queens the work is being carried out by Dr. Schroder and her associates. The following report represents the tabulated summary of the Schick test on more than 150,000 schoolchildren in Manhattan and the Bronx.

A. The Schick Test and Control Test: Dilutions for the Test

The Schick test was made on the right forearm of each child and the control test with heated toxin on the left forearm. The reactions were read from the third to the seventh day after the test, the day of reading depending on how the work could be arranged in the schools.

We consider the proper dilution of the toxin one of the most important elements in the accuracy of the test. When large numbers of children are to be tested, the use of unheated bulk toxin for the Schick test and of heated bulk toxin for the control is recommended as the most accurate and economical method for carrying on the work. It is practically the only way to prepare a large amount of a *uniform* toxin dilution and to be certain of the accuracy of the results as regards one of the essential elements in the test. From an economical point of view this method is most important as we can apply the Schick test in this way to more than 3,000 children, and the expense for the material used will amount to less than one dollar. If proper precautions are taken in making these dilutions as recommended in a previous publication,[1] there will be no danger that dilutions that are too toxic will be prepared. Certainly, health officers, school physicians and general practitioners that have to test large numbers of children should be able to make the proper dilutions without the fear of any unnecessary risks.

B. Susceptibility to Diphtheria by Age Periods

In Table 1 and in the succeeding tables the percentages reported are significant in that they are based upon large numbers of tested individuals. Statistics regarding susceptibility and immunity to diphtheria and conclusions to which they lead should be founded on tests that are accurately carried out on many individuals. Conclusions based on a small number of tests are frequently liable to be misleading.

Table 1 gives a summary of the Schick tests carried out in the public and parochial schools in Manhattan and the Bronx. It shows the susceptibility by age periods of more than 150,000 children in a mixed population, as represented in the schools of New York. It is important to note that

TABLE 1.

THE SCHICK TEST IN THE PUBLIC AND PAROCHIAL SCHOOLS OF NEW YORK (MANHATTAN AND THE BRONX)

Age, Years	Total No. of Children Tested	Schick Positive Reactions	Schick Negative Reactions	Percentage of Schick Positive Reactions
5- 6	4,809	2,902	1,907	**60.3**
6- 7	13,754	6,944	6,810	**50.4**
7- 8	16,180	7,042	9,138	**43.5**
8- 9	17,126	6,278	10,848	**36.6**
9-10	18,065	5,825	12,240	**32.2**
10-11	18,057	5,308	12,749	**29.3**
11-12	17,994	5,075	12,919	**28.2**
12-13	16,258	4,327	11,931	**26.6**
13-14	14,138	3,271	10,867	**23.1**
14-15	9,650	1,906	7,744	**19.7**
15-16	4,861	869	3,992	**17.8**
16-17	369	68	301	**18.4**
Total	151,261	49,815	101,446	**32.9**

more than 60 per cent. of the children gave a positive Schick reaction upon entering school.

Table 2 shows the susceptibility to diphtheria of a large group of children between 6 and 12 months and between 1 and 14 years of age. In Group A are the children that were tested at the baby health stations. They represent a poorer class of the population. It is interesting to note that even in this group of the population there is a very high percentage of positive Schick reactions in children under 1 year, especially from 8 to 12 months of age. The susceptibility continues very high up to 3 years of age. It is probable that even in the poorer classes of the population the very young children are kept fairly well separated, and exposure to repeated infection is thus to a certain extent avoided. The percentage of positive reactions diminishes very rapidly after the age of 7 years.

TABLE 2.

GROUP A: THE SCHICK TEST IN BABY HEALTH STATIONS AND PLAY GROUNDS (MANHATTAN AND THE BRONX)

Age	No. of Children Total Tested	Schick Positive Reactions	Schick Negative Reactions	Percentage of Schick Positive Reactions
6- 7 months	53	30	23	56.6
7- 8 months	41	26	15	63.4
8- 9 months	62	52	10	83.8
9-10 months	58	54	4	93.1
10-11 months	61	53	8	87.0
11-12 months	34	31	3	91.1
1- 3 years	1,727	1,438	289	83.2
4- 6 years	1,328	779	549	58.6
7-10 years	903	330	573	36.5
11-14 years	235	53	182	22.5
Total	4,502	2,846	1,656	63.2

GROUP B: THE SCHICK TEST IN PUBLIC SCHOOL CHILDREN OF KINDERGARTEN AND 1A CLASSES

5- 6 years	327	253	64	77.15
6- 7 years	933	632	301	67.73
Total	1,260	885	365	70.25

TABLE 3.

RESULTS OF SCHICK TEST AT PUBLIC SCHOOL 52 (MANHATTAN)

Age	Total No. of Children Tested	Schick Positive Reactions	Schick Negative Reactions	Percentage of Schick Positive Reactions
5- 6 years	19	16	3	84.21
6- 7 years	69	57	12	82.61
7- 8 years	114	83	31	72.81
8- 9 years	91	69	22	76.26
9-10 years	83	59	24	71.09
10-11 years	60	35	25	58.33
11-12 years	64	44	20	68.75
12-13 years	46	31	15	67.39
13-14 years	42	22	20	52.38
14-15 years	18	11	7	61.11
15-16 years	10	5	5	50.00
Total	616	432	184	70.13

In Group B the table gives by contrast the results in a series of twelve schools in which more than 77 per cent. of the children between 5 and 6 years of age had shown a positive reaction. These schools are located in the less congested sections of the city and attended by children of the more well-to-do. In this group are included a few schools in which from 85 to 90 per cent. of the children in the kindergarten and 1 A classes gave a positive Schick reaction. Table 3 gives the findings in one of these schools.

These figures emphasize the high proportion of susceptible children on admission to school and should be remembered in connection with the practical work of immunizing them against diphtheria. When too many difficulties are in the way of giving the preliminary Schick test to these young children the test could be omitted and the children simply injected with the three doses of toxin-antitoxin.

Table 4 gives the results of a more detailed study of the Schick tests on 60,100 children, which represent a part of the tests in Table 1. In this table the reactions are classified as positive and positive-combined among the non-immunes, and as negative and negative-pseudo among the immunes. The table brings out the following two interesting and important facts:

1. A considerable number of children of school age show negative-pseudo reactions. These reactions should be identified with accuracy by means of the control test. The mistake will thus be avoided of interpreting them as positive Schick reactions with the resulting error of giving the children unnecessarily the injections of toxin-antitoxin. It is in this very group that the toxin-antitoxin produces the most marked local and constitutional symptoms.

2. The negative-pseudo reactions were more than two and one-half times as frequent among the immunes as the positive-combined reactions among

the nonimmunes. This is additional evidence that exposure to infection with the diphtheria bacillus is an important factor in the development of the so-called "natural" antitoxic immunity. Such exposure produces not only an antitoxic immunity, but also seems to sensitize many individuals to the protein substance of the diphtheria bacillus. This sensitization appears in the form of negative-pseudo reactions in the Schick test and control test.

TABLE 4.

THE SCHICK TEST IN THE PUBLIC SCHOOLS OF NEW YORK (MANHATTAN AND THE BRONX): SUSCEPTIBILITY TO DIPHTHERIA BY AGES

Age Years	Total Tested	Nonimmune			Immune			Percentage of Nonimmune			Percentage of Immune		
		Schick Positive Reactions	Positive-Combined Reactions	Total Schick Positive Reactions	Schick Negative Reactions	Negative-Pseudo Reactions	Total Schick Negative Reactions	Of Total Tested	Percentage of Positive Combined Reactions		Of Total Tested	Percentage of Negative-Pseudo Reactions	
									Of Total Tested	Of Total Schick Positive		Of Total Tested	Of Total Schick Negative
4– 5	30	21	0	21	8	1	9	70.0	0.0	0.0	30.0	3.0	11.0
5– 6	1,575	975	21	996	508	71	579	63.2	1.3	2.0	36.7	4.0	12.0
6– 7	5,377	2,586	61	2,647	2,393	337	2,730	49.2	1.0	2.0	50.8	6.0	12.0
7– 8	6,130	2,525	112	2,637	3,010	483	3,494	43.0	2.0	4.0	57.0	8.0	14.0
8– 9	6,539	2,176	187	2,363	3,484	692	4,176	36.1	2.8	8.0	63.9	10.0	16.0
9–10	7,071	2,029	125	2,224	3,802	1,045	4,847	31.4	2.7	9,0	68.6	14.0	21.0
10–11	7,205	1,867	197	2,064	3,997	1,164	5,141	28.6	2.7	9.0	71.4	16.0	22.0
11–12	7,344	1,901	222	2,123	4,063	1,158	5,221	28.9	3.0	10.0	71.1	16.0	22.0
12–13	6,405	1,608	212	1,820	3,748	837	4,585	28.4	3.0	11.0	71.6	13.0	18.0
13–14	6,252	1,188	227	1,415	3,466	1,371	4,837	22.6	3.0	16.0	77.4	22.0	28.0
14–15	4,147	669	123	792	2,520	832	3,352	19.1	3.0	15.0	80.9	20.0	25.0
15–16	1,760	270	50	320	1,068	372	1,440	18.1	3.0	16.0	81.9	21.0	26.0
16–17	247	38	11	49	154	44	198	19.8	4.0	23.0	80.1	18.0	22.0
17–18	18	2	0	2	14	2	16	11.1	0.0	0.0	88.9	11.0	12.0
Total	60,100	17,855	1,618	19,473	32,235	8,409	40,624	32.6	2.7	8.3	67.4	14.0	20.7

The racial factor may also be noted in these tables.[1] The schools in which there is the highest percentage of positive Schick reactions are attended largely by native born children of American parents. The schools showing the lowest percentage of positive Schick reactions are those attended to a great extent by children of Italian parents. The other schools with percentages varying between these extremes are attended by children of more recently immigrated Jewish, Russian, Polish, Czechoslovacian, Irish parents, etc., one or two of these nationalities frequently predominating in some of the schools. A table giving the percentage of positive Schick reactions among children in parochial schools is also interesting in showing that overcrowding and repeated exposure to infection are really more important than race as factors in natural immunity to diphtheria. The three schools in the Bronx in which the children gave the highest percentage of positive Schick reactions are attended largely by children of Irish extraction. On the other hand, some of the schools in Manhattan which show a very low percentage of positive reactions, as for instance St. Bernard's, are also attended mostly by children of Irish parentage. It is significant to note that the school with the lowest percentage of positive Schick reactions, St. Patrick's, is located in the most congested section of the city, in which the population is nearly all Italian.

The results stated in these tables correspond closely to those that were reported in a previous communication in a series of forty-four schools in Manhattan and the Bronx.

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[1] See original paper.

Table 5 shows the very high percentage of positive Schick reactions in public schools located in small suburban towns, attended to a large extent by children of a more well-to-do class. Many of those that gave negative reactions were children of foreign parents recently settled in town.

TABLE 5

THE SCHICK TEST IN THE PUBLIC SCHOOLS OF METUCHEN, N. J.; CALDWELL, N. J.; VERONA, N. J., AND CEDAR GROVE, N. J.

Age, Years	Nonimmune			Immune			Total Tested	Percentage of Schick Positive Reactions
	Schick Positive Reactions	Positive-Combined Reactions	Total Schick Positive Reactions	Schick Negative Reactions	Negative Pseudo Reactions	Total Schick Negative Reactions		
5–6	62	0	62	7	0	7	69	**89.8**
6–7	73	0	73	11	1	12	85	**85.8**
7–8	82	0	82	13	1	14	96	**85.4**
8–9	93	0	93	22	2	24	117	**79.5**
9–10	75	0	75	24	2	26	101	**74.2**
10–11	61	0	61	21	9	30	91	**67.0**
11–12	74	1	75	19	4	23	98	**76.5**
12–13	62	2	64	20	4	24	88	**72.7**
13–14	64	2	66	17	7	24	90	**73.3**
14–15	22	1	23	11	2	13	36	**63.9**
15–16	41	2	43	10	4	14	57	**75.4**
16–17	39	1	40	11	5	16	56	**71.4**
Total	748	9	757	186	41	227	984	**76.9**

Table 6 gives the results of the Schick test in four public schools located in Union Hill, N. J. The school children can be divided fairly evenly into two groups, those of American born and those of foreign born parents. Among the latter we found a much higher proportion of negative Schick reactors.

D. Susceptibility to Diphtheria by Sex

In our previous work with the Schick test, covering a period of more than nine years, we have generally found a larger number of positive reactions among females than among males.

TABLE 6.

THE SCHICK TEST IN THE PUBLIC SCHOOLS OF UNION HILL, N. J.

Age, Years	Schick Positive Reactions	Schick Positive Combined Reactions	Total Schick Positive Reactions	Schick Negative Reactions	Schick Negative Pseudo Reactions	Total Schick Negative Reactions	Total Tested	Percentage of Schick Positive Reactions
5–6	46	0	46	22	1	23	69	**66.66**
6–7	49	1	50	42	3	45	95	**52.63**
7–8	59	4	63	60	9	69	132	**47.72**
8–9	55	5	60	55	14	69	129	**46.51**
9–10	44	5	49	62	22	84	133	**36.84**
10–11	43	6	49	59	30	89	138	**35.50**
11–12	40	5	45	63	31	94	139	**32.37**
12–13	35	7	42	73	30	103	145	**29.00**
13–14	56	9	65	77	53	130	195	**33.33**
14–15	46	10	56	46	30	76	132	**42.42**
15–16	14	7	21	24	11	35	56	**37.50**
16–17	6	0	6	6	2	8	14	**42.85**
Total	493	59	552	589	236	825	1,377	**40.00**

Table 7 shows a definitely higher percentage of positive Schick reactions among girls than among boys. Among the girls there is also a higher proportion of negative-pseudo and positive-combined reactions.

TABLE 7.

COMPARISON OF SUSCEPTIBILITY TO DIPHTHERIA BETWEEN BOYS AND GIRLS IN NEW YORK PUBLIC SCHOOLS (PUBLIC SCHOOLS 188, 39, 83, 159, MANHATTAN)

Age, Years	Boys		Girls		Percentage of Schick Positive Reactions		Percentage of Negative-Pseudo Reactions of Immunes		Percentage of Positive-Combined Reactions of Nonimmunes	
	Total	Schick Positive Reactions	Total	Schick Positive Reactions	Boys	Girls	Boys	Girls	Boys	Girls
5– 6......	74	35	60	27	46.0	45.0	17.5	21.2	0	0
6– 7......	195	74	207	84	37.8	40.6	8.2	24.4	4.1	1.0
7– 8......	248	83	244	93	33.4	38.1	11.5	23.2	3.6	5.3
8– 9......	277	76	219	68	27.4	31.5	15.0	22.2	5.2	11.8
9–10......	393	86	281	82	21.9	39.2	21.5	30.6	7.0	15.8
10–11......	675	147	264	62	21.8	23.4	24.2	38.1	12.7	16.1
11–12......	782	153	299	72	19.5	24.1	26.8	31.7	18.3	15.3
12–13......	783	116	479	99	14.8	20.7	26.5	36.0	14.6	21.2
13–14......	740	91	497	100	12.3	20.1	26.6	35.7	19.7	23.0
14–15......	500	65	361	67	13.0	21.3	27.6	29.5	23.1	19.4
15–16......	224	17	155	21	7.5	13.4	18.3	36.5	0.0	23.3
16–17......	31	2	21	3	6.4	14.3	27.6	33.3	0.0	33.3
Total....	4,922	945	3,087	778	19.2	25.3	23.7	32.3	11.9	14.2

In Table 8 are given the results of the Schick test on more than 6,000 adult inmates of an insane asylum. The table brings out the fact that even among those who are far advanced in years there is definite though small percentage of positive Schick reactors. It shows that repeated exposure even

TABLE 8.

THE SCHICK REACTION IN ADVANCED AGE GROUPS (MANHATTAN STATE HOSPITAL)

Age, Years	Schick Positive Reactions	Schick Positive Combined Reactions	Total Schick Positive Reactions	Schick Negative Reactions	Schick Negative Pseudo Reactions	Total Schick Negative Reactions	Total Tested	Percentage of Schick Position Reactions
Males—								
10–20...........	12	1	13	57	18	75	88	14.7
20–30...........	47	8	55	551	99	650	705	7.8
30–40...........	45	11	56	580	110	690	746	7.5
40–50...........	28	2	30	418	69	487	517	5.8
50–60...........	21	1	22	292	20	312	334	6.5
60–70...........	5	0	5	142	4	146	151	3.3
Over 70.........	2	0	2	59	1	60	62	3.2
Total...........	160	23	183	2,099	321	2,420	2,603	7.0
Females								
10–20...........	9	2	11	60	9	69	80	13.7
20–30...........	76	15	91	376	81	457	548	16.6
30–40...........	89	13	102	514	126	640	742	13.7
40–50...........	58	12	70	546	87	633	703	9.9
50–60...........	34	3	37	466	83	549	586	6.3
60–70...........	30	1	31	446	34	480	511	6.6
Over 70.........	8	0	8	107	4	111	119	6.7
Total...........	304	46	350	2,515	424	2,939	3,289	10.0

over a long period of years does not produce an antitoxic immunity in certain individuals. These figures help to explain why it is difficult to develop an active immunity in a small percentage of individuals even with several series of toxin-antitoxin injections. There is a partial or nearly total inability of the tissues in some people to respond to the immunizing effects of certain antigens. This resistance is probably hereditary in character.

The table also shows a higher percentage of positive Schick reactions among adult females than among males.

E. "Natural" Immunity to Diphtheria

In the preceding tables we have seen some of the important factors active in the development of spontaneous immunity to an endemic infectious disease. These factors consist of crowding and repeated exposure to infections with the diphtheria bacillus. In cities this organism is constantly present. From 2 to 3 per cent. of the inhabitants are carriers of virulent diphtheria bacilli. The high percentage of positive Schick reactions among the children of the more well-to-do and the low percentage among those of the poorer classes show the importance of overcrowding in the development of immunity. In the smaller communities and especially in rural sections where the people live more or less segregated, the percentage of positive Schick reactions is very high. In rural schools we have seen from 85 to 90 per cent. of positive Schick reactions. This is true not only for children but also for adults. In various city hospitals we have tested groups of nurses, many of whom had only recently come to the city from smaller communities, and have found that among them from 50 to 75 per cent. gave a positive reaction to the Schick test. Similar observations were reported to me in a personal communication by Dr. Charles W. Kidder, District Health Officer of Woodstock, Vermont. He gave the Schick test to 2,030 children and found in rural towns, with a very high percentage of native born children, as high as 96 per cent. of positive reactors. In the larger villages on the railroad and in villages containing a large percentage of foreign population, he obtained correspondingly fewer positive Schick reactors, the lowest percentage being 60. Of eighty-seven teachers between the ages of 20 and 60 years, 82 per cent. gave a positive reaction.

Table 9 is an interesting study of the effect of close contact in an institution. It gives the results of the Schick test in an institution in which most of the children came from New York City. Under "Institution" children are included those that have been at the institution three months or longer; under "Preventorium" children those who have been there less than three months.

Among the "Preventorium" children, twenty-six of a total of seventy-eight, or 33.3 per cent., gave a positive Schick reaction. This is in striking contrast to the "Institution" children of whom only twenty-eight of a total of 520, or 5.4 per cent., gave positive reactions. Even among the twenty-eight positively reacting "Institution" children, fully twenty had been in the institution only from three months to two years. Frequent contact in the institution explains these results.

Dudley has recently reported the results of the Schick test in an institution in which diphtheria had been prevalent and had resulted in a large number of carriers. This study furnishes additional evidence that exposure to infection with the diphtheria bacillus is an important factor in the development of contact or so-called "natural" immunity.

TABLE 9.

THE SCHICK TEST AT ST. AGATHA'S HOME, NANUET, N. Y.

	Institution Children						Preventorium Children (Less Than Three Months in the Institution)			
Age, Years	Total Tested	Schick Negative Reactions	Negative-Pseudo Reactions	Total Immune	Schick Positive Reactions	Percentage of Schick Positive Reactions	Total Tested	Schick Negative Reactions	Schick Positive Reactions	Percentage of Schick Positive Reactions
2– 3..........	8	4	0	4	4	50.0	0	0	0	0
3– 4..........	13	8	0	8	5	38.5	0	0	0	0
4– 5..........	21	16	0	16	5	23.8	6	2	4	66.6
5– 6..........	22	15	1	16	6	27.3	6	4	2	33.3
6– 7..........	53	42	2	44	9	17.0	7	4	3	43.0
7– 8..........	36	25	6	31	5	13.9	16	9	7	43.7
8– 9..........	44	35	7	42	2	4.6	9	6	2	33.3
9–10..........	54	44	8	52	2	3.8	11	10	1	9.0
10–11..........	63	59	3	62	1	1.6	9	6	3	33.3
11–12..........	59	46	10	56	3	5.0	8	6	2	25.0
12–13..........	67	51	13	64	3	4.5	2	1	1	50.0
13–14..........	50	37	9	46	4	8.0	3	3	0	0.0
14–15..........	38	30	6	36	2	5.3	1	1	0	0.0
15–16..........	15	11	4	15	0	0.0	0	0	0	0.0
Total........	543	423	69	492	51	9.4	78	52	26	33.3
Total*.......	520	...	...	492	28†	5.4	...	...	...	33.3

* Excluding twenty-three children giving a positive reaction who had been less than three months in the institution.

† Of the twenty-eight children giving a positive reaction, twenty had been in the institution between three months and two years.

Without going too far astray one could probably generalize from "natural" immunity against diphtheria to "natural" immunity against some of the other infectious diseases. Such immunity is seen in a large group of city born and city bred people who have never had these infections. Russell has drawn our attention to the fact that, during the recent war, epidemics of infectious diseases swept, as a rule, through camps in which the soldiers were recruited from the smaller towns and villages and from rural sections of the states. Many city children are often found to be relatively immune to some of these infectious diseases although they give no history of having had the infections. Herrman's observations on immunity to measles also bear on this point. It has, of course, been often assumed that repeated exposure to infectious diseases, or to the carriers of their viruses or bacteria, is the chief factor in "natural" immunity; but so far it is only in diphtheria that we have been able to demonstrate this with any degree of scientific accuracy.

Summary and Conclusions

1. More than 150,000 children have been given the Schick test during the last two years in the public and parochial schools of the boroughs of Manhattan and the Bronx.

2. A high percentage of positive Schick reactions is found among the children in the entering classes in the kindergarten and 1 A grades. This varies in different schools from 60 to 85 per cent. The toxin-antitoxin injections could, therefore, be given to these young children without the preliminary Schick test.

3. The schools located in the less congested sections of the city and attended by children of the more well-to-do show, as a rule, from 100 to 200

per cent. more Schick positive reactors than those located in the crowded parts and attended by children of the poorer classes.

4. Repeated exposure to infection with the diphtheria bacillus is probably the most important factor in the development of "natural" immunity to this disease. Racial and hereditary family factors, although less important, also play a rôle.

5. Girls show a somewhat larger percentage of positive Schick reactions than boys. They also show more negative-pseudo and positive-combined reactions. This holds true also for adults.

6. The high percentage of positive Schick reactors among young children of preschool age may, under certain conditions, render it advisable to simplify the procedure of immunization by omitting the preliminary Schick test. The children are simply injected with three doses of toxin-antitoxin. Under these circumstances, however, the children should not be pronounced immune to diphtheria until a supplementary Schick test, performed six months later, proves negative.

7. Diphtheria is the foremost fatal disease in children from 3 to 5 years of age, and ranks third among the fatal diseases of children from 1 to 3 years of age. Only diarrhea and pneumonia precede diphtheria in the mortality tables. The dangerous laryngeal form occurs in the young children. It is for these reasons that physicians should urge parents to take advantage of these newer methods to protect their young children against diphtheria. In fact, active immunization against diphtheria should become as much of a routine for all children between 6 months and 6 years as vaccination is against smallpox today.

For bibliography see the original article.

COMPARATIVE MERITS OF NEW AND OLD PREPARATIONS OF DIPHTHERIA TOXIN-ANTITOXIN

Results of Preliminary Tests with Toxoid

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When the New York City Health Department laboratory workers first undertook to estimate the value of toxin-antitoxin in the practical immunization of children against diphtheria in 1914, it was essential to utilize an effective preparation. The ingredients of importance in such a preparation other than the antitoxin are the specific toxin that causes the production of antitoxin and the nonspecific products of bacterial growth and dissolution occurring in the cultures in which the toxin is produced. It is these nonspecific products that cause the local disturbance in sensitive persons. The antitoxin should be added in an amount just sufficient to lessen the excessive avidity of the toxin for the tissues and so prevent painful reaction, but the amount of antitoxin should not be enough to rob the toxin of its immunizing effect.

For a number of years previously, numerous animal tests had been carried out by Park with a mixture containing three L plus doses of toxin to each cubic centimeter, and sufficient antitoxin to reduce the toxicity so that an injection of 1 c.c. caused paralysis in the guinea-pig in about twenty-five days.

As we had studied this preparation and knew it to be efficient and safe, we determined to use it for the immunizing injections. The results were in every way satisfactory except for the rather excessive local reaction. This mixture became our standard preparation. However, with the increasing utilization of toxin-antitoxin not only in New York City but also throughout the country, we felt constrained to endeavor to remove as much as possible of the irritating substances in the mixture.

Dr. Banzhaf of our laboratory attacked this problem, but neither he nor other chemists have been able to separate the useless and annoying bacterial substances in the culture fluids from the valuable specific toxin. Despairing of success in eliminating the extraneous proteins from the specific toxin, we attempted to achieve our object by lessening the amount of both toxin and antitoxin in the mixture and so also the amount of the proteins. This was suggested to us by the reports of the Schick retests by Zingher on a number of children in the borough of Manhattan, and by Schroder on children in the borough of Brooklyn. A toxin of double the standard strength had been used on these children. The immunizing results, instead of exceeding those obtained with the standard preparation, were merely similar. A doubling the amount of toxin in the dose did not improve the effectiveness of the mixture, it seemed reasonable to try the effect of reducing the amount of toxin. We therefore tried mixtures in which the amount of toxin was one third, one sixth, one thirtieth and one sixtieth of the standard dose. The preparation (0.1 L + toxin) containing one thirtieth of the original toxin was finally selected as probably the best. A preliminary report of the observations on 490 children on whom this mixture was employed by Schroder was given at the meeting of the American Public Health Association[1] in October, 1922. The results are shown in Tables 1 and 2.

Because of these favorable results, it was decided to make the one-thirtieth (one-tenth L plus toxin) preparation the standard, instead of the three L plus mixture. Since then no other mixture has been used in our work in immunization except when we wished to make comparisons. The results of a comparative test in adults are given in Table 3.

TABLE 1.

THE ANTITOXIN DEVELOPMENT PRODUCED BY THREE INJECTIONS OF MIXTURES* HAVING DIFFERENT AMOUNTS OF TOXIN PARTIALLY NEUTRALIZED BY ANTITOXIN

Amount of original toxin in 1 cc. of Mixture	Number of children receiving 3 injections	Per-Cent of nonimmunes shown to be immune on Schick retest four months later
0.1 L+† (4 lethal doses)......................	490	90
0.5 L+ (20 lethal doses)......................	304	95
3.0 L+ (120 lethal doses)....................	318	92
5.0 L+ (200 lethal doses)....................	487	85

*All mixtures caused severe paralysis in guinea-pigs followed usually by death in eighteen days among those animals that received 1 c.c.; with 5 c.c. doses, death followed in ten days.

†The mixture is made by adding three-fourths unit of antitoxin to one L plus dose of toxin. The toxin and antitoxin should be diluted in cold water and the two solutions mixed immediately. If the toxin is diluted in water at room temperature, it deteriorates rapidly.

TABLE 2.

COMPARISON AS TO THE AMOUNT OF LOCAL AND CONSTITUTIONAL REACTION CAUSED BY THE NEW AND OLD PREPARATIONS IN CHILDREN

	New Preparation 0.1 L+* %	Old Preparation 3 to 5 L+ %
No local reaction................................	25	0
Slight local reaction............................	64	41
Moderate local reaction........................	11	37
Marked local reaction..........................	0	22
Among those showing marked reactions there was a rise of temperature of from 1 to 3 degrees F., and other constitutional symptoms in............	0	6

*If the 0.1 L plus preparation is underneutralized more than the amount advised, there will be a local reaction from the excess of toxin.

The immunizing results in adults with the one-tenth L plus and the three L plus preparations were similar to those previously obtained in children (Table 1). The local reactions to both preparations were somewhat more severe than with similar preparations in the children (Table 2), but the amount of local reaction following injections of the one-tenth L plus mixture was strikingly less than that following the injections of the three L plus mixture. The slightly lesser immunity response of the one-tenth mixture is attributed to the fact that the toxin was slightly less neutralized. One group of adults received three doses of only 0.5 c.c. each instead of 1 c.c. of the mixture. Those individuals who received the smaller injections had nearly as much local reaction without as great an immunity response.

Local and Immunity Reactions Produced by Toxin and by Toxoid

In the first experiments on antitoxin production in animals by von Behring and his associates, the diphtheria toxin was modified by exposure to iodin so as to reduce its toxicity. The action of the iodin or of other agents that cause a lessening of the toxicity and reduce the toxin to a form called toxoid produced an effect on the toxin somewhat similar to the action of slight amounts of antitoxin. We thought it would be of great interest to compare the results of such injections with those obtained by the injection of the toxin-antitoxin. It happened that at this time we had a large number of adults to immunize. This enabled us to use the different preparations under comparable conditions. We utilized a toxin that had been stored after being diluted with salt solution and had thus been reduced to toxoid. This was carefully tested in guinea-pigs. We arranged also to give a small group three injections of standard toxin at the same time. The amount of toxin chosen was that used in the Schick test. Through a misunderstanding, the first dose given was four times this amount. The results of these various preparations are shown in Table 3.

The local reaction of an appreciable dose of standard toxin alone was too severe to make it an acceptable preparation for general use. On the other hand, as shown in Table 3, the deteriorated toxin (toxoid) gave a fairly satisfactory immunizing response with remarkably little local reaction. We hope in the near future to carry on further tests with the diphtheria toxoid preparations. It is evident that fresh toxin must either be modified by antitoxin or partially broken down by some chemical or physical agency, so as to lessen the avidity with which it attacks the tissues; otherwise, the local reaction of the tissues is so severe that the amount of toxin injected has to be reduced to a dose so small that the immunity response in those who have no antitoxin will be very much less than when the usual dose of toxin-antitoxin or toxoid is used.

TABLE 3.

LOCAL REACTION ON SECOND DAY AND IMMUNITY RESPONSE AT THE END OF THREE AND ONE-HALF MONTHS FROM TOXIN-ANTITOXIN, UNMODIFIED TOXIN AND TOXOID*

Preparation Used	Three doses of, cc.	Adults tested	Severity of Local Reaction					Immunity response after 3½ months Schick negative %
			Very marked, %	Marked, %	Moderate, %	Slight, %	None, %	
Toxin-antitoxin:†								
3.0 L+ in 1 c.c.	1	261	13	36	22	12	17	87
0.1 L+ in 1 c.c.	1	117	..	10	20	44	26	83
0.1 L+ in 1 c.c.	0.5	86	..	8	23	45	24	73
Freshly diluted standard toxin without antitoxin.								
0.15 M. L. D. in 1 c.c. 1st dose	1	29‡	3.5‡	24‡	45‡	24	3.5	41
0.025 M. L. D. in 1 c.c. (2d and 3d doses)	1	29	..	4	8	25	62	
Toxoid § (old and much deteriorated toxin)	1	211	..	3	10	37	50	70

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* This table was given in the preliminary report read at the meeting of the Association of Pathologists and Bacteriologists in March, 1923.

† Average toxicity when injected in guinea-pigs weighing 250 gm.: 3 L plus in 1 c.c. = death in 23 days; 0.1 L plus in 1 c.c. = death in 30 days.

‡ The local reaction continued to increase after the second day instead of decreasing as it does with properly standardized toxin-antitoxin or toxoid.

§ One cubic centimeter caused late paralysis in guinea-pigs.

IMPORTANCE OF A SUITABLE DEGREE OF NEUTRALIZATION OF TOXIN IN TOXIN-ANTITOXIN MIXTURE

There has been considerable difference of opinion among investigators on this subject. Park and Banzhaf will soon make a final report on a large number of observations obtained from guinea-pig experiments. We report here the result of the retests on more than 2,000 children, on some of whom preparations of standard toxicity were used and on others preparations of somewhat insufficient toxicity (Table 4).

TABLE 4.

IMMUNITY RESULTS IN CHILDREN AFTER THREE INJECTIONS OF TOXIN-ANTITOXIN MIXTURES OF DIFFERENT DEGREES OF NEUTRALIZATION

Preparation No.	Toxicity in guinea-pigs Dose	Result	Number of children injected	Immunity response after 4 to 6 months Schick negative, per cent.
1	1 c.c.	Death in about 17 days	131	92
	5 c.c.	Death in 4 to 5 days		
2	1 c.c.	Death in about 17 days	269	92
	5 c.c.	Death in 5 to 8 days		
3	1 c.c.	Death in about 21 days	796	91
	5 c.c.	Death in 5 to 6 days		
4	1 c.c.	Marked paralysis of animal followed by recovery	608	87
	5 c.c.	Death in about 17 days		
5	2 c.c.	Marked paralysis of animal followed by recovery	137	85
	5 c.c.	Death in about 22 days		
6	2 c.c.	Marked paralysis of animal followed by recovery	763	76
	5 c.c.	Death in about 25 days		
			2,704	

TABLE 5.

COMPARISON OF SCHICK RETEST RESULTS AT DIFFERENT INTERVALS AFTER IMMUNIZATION

Interval	Number of retests	Per cent. immune
3 to 3½ months	579	82.7
4 months	1,427	84
5 months	1,302	86.7
6 months	1,059	88
	4,367	

It is noticeable that, while the ideal preparations gave the best results, those preparations approaching considerably nearer to complete neutralization still yielded fair results. Therefore, although it is important to have preparations of a proper degree of toxicity, it is valuable to know that less toxic preparations will give in the majority of children sufficient antitoxic immunity response.

TABLE 6.

COMPARATIVE INTENSITY OF LOCAL REACTION TWENTY-FOUR AND FORTY-EIGHT HOURS AFTER TOXIN-ANTITOXIN INJECTIONS

Greatest intensity of reaction	Total number of persons observed	Number having greatest intensity of reaction at		
		24 hours	48 hours	Same at 24 and 48 hours
Very marked	23	..	4	19
Marked	31	6	9	16
Moderate	15	4	6	5
Slight	21	..	20	1
Totals	90	10	39	41

TIME INTERVAL BETWEEN INJECTION OF TOXIN-ANTITOXIN AND DEVELOPMENT OF ANTITOXIN IN SUFFICIENT AMOUNT TO INSURE IMMUNITY

It is necessary to make a retest for the Schick reaction if we desire to know positively whether the immunizing injections have given complete protection. Although it is desirable to make these retests as soon as possible, we defeat our purpose if they are made before the majority of the children have had time to react. The results obtained in about 4,000 retested children are given in Table 5.

These results indicate that although longer periods give somewhat more favorable results, retests may be made satisfactorily at any time after three months. The children tested at these intervals had received identical toxin-antitoxin preparations.

While the observations reported in this paper were being made, certain facts were noted that are of considerable clinical interest. These are the interval of time after the injection at which the intensity of the local toxin-antitoxin reaction is at its greatest, and the duration of the reaction. These observations are given in Tables 6 and 7.

TABLE 7

COMPARATIVE INTENSITY OF THE LOCAL REACTION AT END OF TWO, FIVE AND SIXTEEN DAYS, IN THREE HUNDRED CASES ASSEMBLED EQUALLY FROM PERSONS SHOWING MARKED, MODERATE AND SLIGHT REACTIONS

Time of inspection	Severity of reaction			
	Marked	Moderate	Slight	None
End of 2 days	100	..	..	..
End of 5 days	4	31	19	46
End of 16 days	..	..	7	93
End of 2 days	..	100	..	..
End of 5 days	..	11	26	63
End of 16 days	..	..	7	93
End of 2 days	..	..	100	..
End of 5 days	..	..	12	88
End of 16 days	..	..	..	100

As shown in the table, the local reaction subsides gradually after reaching its height on the first or second days.

CONCLUSIONS

The new preparation of toxin-antitoxin, with its smaller amount of toxin, possesses a distinct advantage over the earlier preparation recommended by us, in that the local inflammatory reaction sometimes developing after an injection is less severe and less frequent. Indeed, it is almost negligible in your children and infants. The two preparations have equal immunizing value.

The toxin-antitoxin mixture should not be too toxic; that is, too much underneutralized; if it is, the local reaction is disturbing. It should not be too nearly neutralized by antitoxin, for then the immunizing results are less. Safe limits as to toxicity are that 1 c.c. should not produce death in the guinea-pig in less than fifteen days on the average, and 2 c.c. should not fail to produce marked paralysis.

Antitoxin is added to diphtheria toxin solely for the purpose of robbing it of its irritant action on the local tissues and thus allowing a larger dose of the modified toxin to be injected. The same result may be obtained by partially breaking down the toxin to toxoid.

Unmodified standard toxin, such as is used in the Schick test, is so irritating that only minute doses can be used. Even these minute amounts have some immunizing value. Up to the present time, the addition of antitoxin to toxin gives us the best immunizing preparation.

The new preparation of toxin-antitoxin is somewhat less stable than the original preparation, but it is sufficiently stable to be used for six months after it has been properly standardized, if it is kept cool.

The use of the Schick test to determine the immunizing results of the toxin-antitoxin injections should not be approved of before the end of the third month, and preferably not before the end of the fifth month. This is because the immunizing response is a gradual one and requires for its completion a different period of time in each individual.

The local reaction which sometimes follows the toxin-antitoxin injections reaches its height on the first or second day, and then rapidly declines.

For bibliography see the original article.

IMMUNITY RESULTS OBTAINED WITH DIPHTHERIA TOXOID (MODIFIED TOXIN) AND ONE-TENTH L + MIXTURES OF TOXIN-ANTITOXIN IN THE PUBLIC SCHOOLS OF NEW YORK CITY (MANHATTAN AND THE BRONX)

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INTRODUCTION

The large and increasing use of subcutaneous injections of some form of modified toxin for purposes of active immunization, makes it important to utilize this toxin in the form in which it will produce the greatest amount of immunity, with minimum amount of deleterious after effect. It is important to grasp that in any preparation it is the diphtheria toxin or derivatives of it, and nothing else, that immunizes. Ehrlich, in 1895, discovered that when fresh diphtheria toxin deteriorates in strength a peculiar change takes place. The toxicity for the guinea-pig drops faster than its power to neutralize antitoxin. At a certain stage in deterioration the toxin in even large doses no longer causes death in animals, but it still neutralizes a considerable percentage of its original amount of antitoxin. Thus, if the preparation drops in toxicity from 100 minimum lethal dosage to 5 minimum lethal dosage per cubic centimeter, the original power to neutralize 1.0 unit of antitoxin still holds to the extent of neutralizing 0.1 of a unit; that is, while the toxicity has diminished to one-half of 1 per cent., the combining power has diminished only to 10 per cent.

Before Ehrlich had finished his researches, von Behring had already used this deteriorated toxin in his first experiments on the production of antitoxin. He utilized the addition of a small percentage of iodin tetrachlorid (1 : 500) to change the toxin so that he could immunize the animals safely. A little later it was discovered that by adding an amount of antitoxin to the toxin which would neutralize, or nearly neutralize, its toxicity in animals, its immunizing power still remained. The first modification of the toxin is called toxoid, the second toxin-antitoxin. While both of these modifications of toxin continued to be used in the immunization of animals, attention was concentrated more on the toxin-antitoxin, and it thus was the first to be used in the immunization of man. Thus it was that von Behring used it in his injections on human subjects.

When we began the work in New York, we also utilized a preparation of toxin-antitoxin because we were familiar with it. At first it was our view that a large amount of toxin almost completely neutralized would be more effective than a smaller amount less nearly neutralized. We therefore utilized a toxin-antitoxin containing in each dose 3 L + (120 minimum lethal dosage) of toxin and 3½ units of antitoxin. The immunizing results were satisfactory, but the local inflammatory reaction was occasionally severe in those sensitive to the nonspecific substances. We tried a preparation having nearly double the amount of toxin. This, to our surprise, gave no better results.

Comparison Between Old and New Type Mixtures of Toxin-Antitoxin

Table 1 shows that mixtures with the larger number of L + doses were not more efficient than those with the smaller number of L + doses, so long as the mixtures were equally toxic (under-neutralized). In fact, we noted that mixtures with the smaller number of L + doses per cubic centimeter, but showing greater toxicity for the guinea-pig, gave better immunity results than those with the larger number, which were more neutralized. This led one of us (P.) to make use of preparations containing less toxin and finally one containing but one-thirtieth of the amount of toxin, or 0. L + and 0.75 unit of antitoxin was adopted. The results were as satisfactory as with the other preparation, and the local reaction was much less. This has become the standard preparation in this country.

TABLE 1.

COMPARISON BETWEEN DIFFERENT MIXTURES OF TOXIN-ANTITOXIN VARYING IN TOXICITY AND NUMBER OF L + DOSES PER CUBIC CENTIMETER (ZINGHER)

		Action on Guinea-Pig (Tests by Banzhaf)		Results in Children				
Mixture	Composition of Toxin Antitoxin Mixture	Amount injected, C.c.	Result	Number doses	Amount of each dose, cc.	Total number Schick retested	Schick negative	Per cent. negative
41	6 L doses of toxin and 7 units of antitoxin	1.0	Partial paralysis in 10 days; recovery	3	1.0	985	570	57.8
		5.0	Paralysis; death in 15 days					
42	5 L doses of toxin and 5.8 units of antitoxin	0.5	Paralysis; death in 17 to 20 days	3	1.0	3,846	3,356	87.3
		1.0	Paralysis; death in 17 days					
		5.0	Death in 3 days					
43	3 L doses of toxin and 5.8 units of antitoxin	1.0	Paralysis in 16 days; recovery	3	1.0	142	137	95.9
		3.0	Paralysis; death in 17 days					
		5.0	Death in 3 days					

Table 2 shows the antitoxin development after three injections of mixtures having different amounts of toxin and all partially, but equally, neutralized by antitoxin.

TABLE 2.

THE ANTITOXIN DEVELOPMENT PRODUCED BY THREE INJECTIONS OF MIXTURES* HAVING DFFERENT AMOUNTS OF TOXIN PARTIALLY NEUTRALIZED BY ANTITOXIN (SCHROEDER)

Amount of original toxin in 1 cc. of mixture	Number of children receiving three injections	Percent of nonimmunes shown to be immune on Schick retest four months later
0.1 L+ (4 lethal doses)	490	90
0.5 L+ (20 lethal doses)	304	95
3.0 L+ (120 lethal doses)	318	92
5.0 L+ (200 lethal doses)	487	85

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* All mixtures caused severe paralysis in guinea-pigs, followed usually by death in eighteen days among those animals that received 1 c.c.; with 5 c.c. doses, death followed in ten days.

Table 3 shows the mixtures used, the effect of varying doses and of mixtures of varying toxicity on guinea-pigs, and the immunity results in children. It is interesting to note the high percentage of children who became immune with mixtures 69 and 65. The number of retested children is sufficiently large to be representative, and thus avoid the error so frequently associated with small numbers in diphtheria immunity work.

TABLE 3.

COMPARISON OF RESULTS WITH DIFFERENT MIXTURES OF TOXIN-ANTITOXIN CONTAINING FROM ONE-TENTH TO ONE-TWENTIETH L + PER CUBIC CENTIMETER RELATION BETWEEN ACTION ON GUINEA-PIG AND IMMUNIZING EFFICIENCY IN CHILDREN (ZINGHER)

			Action on Guinea-Pig (Tests by Banzhaf)		Results in Children				
Mixture	Number L+ in each cc.	Number M.L.D. in each cc.	Amount Injected, cc.	Result	Number doses	Amount, cc.	Total number Schick retested	Schick negative	Per cent. negative
69	1/10	4	1.0	Partial paralysis; recovery	3	1.0	221	213	96.4
			3.0	Paralysis; death in 25 days					
			5.0	Death in 4 days					
65	1/10	4	1.0	Death in 8 days	3	1.0	900	814	94.0
			5.0	Death in 6 days					
67	1/10	4	1.0	Paralysis; death in 22 days	3	1.0	61	53	86.9
			3.0	Death in 9 days					
			5.0	Death in 3 days					
52	1/10	4	2.0	Paralysis in 21 days; recovery	3	1.0	384	342	89.0
			3.0	Paralysis in 17 days; recovery					
			5.0	Paralysis in 17 days; recovery					
70	1/10	4	1.0	Partial paralysis; recovery	3	1.0	55	48	87.4
			3.0	Paralysis; recovery					
			5.0	Paralysis; death in 21 days					
53	1/10	4	1.0	Paralysis; death in 21 days	3	0.5	28	24	85.7
			3.0	Death in 3-5 days	3	0.25	31	24	77.4
			5.0	Death in 3-5 days					
56	1/20	2	1.0	No local effect; no paralysis; recovery	3	1.0	165	97	59.4
			3.0	Paralysis in 21 days; death in 35 days					

Local and Constitutional Reactions Produced by New and Old Type Mixtures of Toxin-Antitoxin

The local and constitutional reactions obtained with mixtures of toxin-antitoxin are due to two different substances in the mixtures.

(*a*) In the old type mixtures, containing from 3 to 6 L + doses of toxin per cubic centimeter, there was a considerable amount of autolyzed bacillary substance and other kinds of portein derived from the broth culture itself, which gave rise in sensitive persons to the most marked forms of local and constitutional reactions. These reactions were of an anaphylactic type and generally occurred in hypersensitive persons, who had given a negative pseudo reaction or positive combined reaction. Both the local and constitutional reactions reached their height within twenty-four hours, gradually disappeared in from two or three days, although they persisted at times for from four to six days. The local reaction varied from an area of redness and induration the size of a fifty cent piece to an extensive infiltration involving one-half, or more, of the arm. There was pain, tenderness and slight glandular enlargement in the axilla. Fever was present, the temperature ranging from 100 to 104 F. A few of the persons showing simple positive Schick reactions also had slight local and constitutional disturbances, but the more marked reactions to the toxin-antitoxin were only found in those who had shown themselves to be hypersensitive to the proteins of the mixture by their negative pseudo reactions or positive combined reactions.

(*b*) With the new type of mixture (one-tenth L +) the local and constitutional effects due to the reacting protein were to large extent eliminated, but the local effect of the larger amount of free toxin present in some of these preparations became more evident. We noticed, following the subcutaneous injections, especially with mixtures of which 1 c.c. caused death of the guinea-pig in from six to eight days and 3 c.c. in from three to five days, local reactions that were slow in appearing, but much more lasting than the protein reactions. These reactions were characterized by local induration and redness, which gradually faded into a brownish-red area of discoloration. The area had a dry, wrinkled, desquamating appearance and resembled on inspection a large positive Schick reaction. With one of these mixtures we even noticed in a few children a superficial necrosis of the skin. This local reaction was due to a large extent to the free toxic fraction of the mixture.

The majority of the mixtures of toxin-antitoxin used in the schools were less toxic, and corresponded more to the degree of underneutralization given for a standard preparation. With such mixtures the local reactions were only slight and often absent. It was possible, in fact, to inject several hundred school children with these new type mixtures during a day's work without hearing subsequent complaints about sore arms, or the necessity of the children remaining away from school for a day or two. Constitutional symptoms, such as fever, were practically absent. This was noticeable even after injecting the more toxic preparations, and was in striking contrast to the disagreeable effects seen with the 3 to 6 L + mixtures in from 5 to 20 per cent. of school children.

Equally suggestive of the value of toxoid as a good immunizing agent were the results obtained by Zingher with the old type mixtures (from 3 to 5 L + doses per cubic centimeter) of toxin-antitoxin. Table 4 indicates that these mixtures did not have their immunizing value impaired to any extent

TABLE 4.

RESULTS OBTAINED WITH FRESH MIXTURES AS COMPARED WITH OLDER MIXTURES OF TOXIN-ANTITOXIN CONTAINING FROM THREE TO FIVE L + TOXIN PER CUBIC CENTIMETER (ZINGHER)

			Action on Guinea-Pig (Tested by Banzhaf)		Results in Children			
Mixture	Number L + in each cc.	Number M.L.D. in each cc.	Amount injected cc.	Result	Number doses toxin-antitoxin	Total number Schick retested	Schick negative	Per cent. negative
44	..	...	1.0	Death in 6 days	3	1,080	1,047	97.0
Fresh	3	120	5.0	Death in 3 days				
44*	3	120	1.0	Slight local effect; death from pneumonia	3	142	137	96.4
Old	..	...	3.0	Moderate local effect; death in 12 days				
			5.0	Moderate local effect; death in 9 days				
42	5	200	0.5	Paralysis; death in 17-20 days	3	3,846	3,356	87.2
			1.0	Paralysis; death in 17 days				
Fresh	..	...	5.0	Death in 3 days				
42*	5	200	1.0	No local effect; no paralysis, recovery	3	364	361	99.1

* These mixtures were kept at icebox temperature for seven months.

after standing for several months, and losing nearly all their toxicity for the guinea-pig. The slight excess of free toxin being changed into toxoid, these favorable results indicated that toxoid could give rise to antitoxic antibodies and that it was probably an important factor in active immunization. Dissociation of the neutral mixture of toxin-antitoxin after injection need not be considered of any importance, in view of the poor immunizing value of freshly neutralized or overneutralized mixture.

Immunity Results Obtained with Toxoid

The above results suggested that there were possibilities in the toxoid preparation that made it well worth while to submit it to further experimentation. For the time being our attention was mainly centered on the new one-tenth L + preparation of toxin-antitoxin. The article of Glenny and Hopkins, on using a toxoid with antitoxin instead of the toxin-antitoxin mixture regularly used, then appeared. A little later O'Brien, of the Burroughs, Wellcome & Company laboratories, in writing to one of us said that they were getting excellent immunity results in animals with toxoid made by the addition of 0.1 per cent. formaldehyd to toxin. He sent some of this preparation to us and asked if we would try it on human beings. Dr. Banzhaf immediately injected it into guinea-pigs and Dr. Sawhill injected a few nurses at Bellevue Hospital. The guinea-pigs, when retested three and four weeks later showed immunizing results as good, but not better, than with the toxin-antitoxin preparation.

Glenny. Pope and Hopkins, in an as yet unpublished communication entitled "Further Notes on Modification of Diphtheria Toxin," state that the amount of formaldehyde necessary to produce a given effect on a batch of toxin depends greatly on the aminonitrogen content. To a toxin of which the van Slyke nitrogen was 2.8 mg. per 10 c.c., 0.1 per cent. of formaldehyd was added; after keeping for twenty-four hours at a temperature of 37 C., an intradermic reaction was no longer obtained if the toxin was diluted more than 250 times. Another toxin, with a van Slyke figure of 19.6 mg., after similar treatment, gave a reaction when diluted 10,000 times; this toxin needed at least 0.3 per cent. formaldehyde to reduce the toxicity to the same level as that reached by adding 0.1 per cent. to the other toxin. In a given toxin the amount of modification produced by a given concentration of formaldehyd depends on the temperature and time of exposure.

After modification of toxin many of the tests for combining power can no longer be made. If sufficiently modified, the toxin no longer kills the guinea-pig and so the L + dose cannot be determined. Even the reacting dose on the guinea-pig's skin can no longer be estimated if the toxin is completely changed into toxoid. To obtain an indication of the combining power of such a modified toxin that is no longer capable of producing symptoms in a guinea-pig, Glenny, Hopkins and Pope have added an excess of antitoxin, and after standing the mixture for a number of hours, determined by titration with toxin the amount of free antitoxin left in the mixture. By making a similar experiment previously with the same toxin, which had not been modified, they were able to draw some interesting and important conclusions, indicated in Table 5.

In Table 5 the number of units combined represents the difference between the number of units of free antitoxin detected in the mixture and the number of units originally added. This number of units they call the total combining power. They found that the total combining power had not been markedly reduced by changing the toxin into toxoid, but that the affinity of the modified toxin for antitoxin had been greatly reduced. This is indicated in parts 2 and 3 of Table 5. Leaving the toxoid in contact with the antitoxin for only four hours, as was done in the case of the unmodified toxin, the total combining power was found to be much less; it required fully forty-eight hours for the complete saturation of the toxoid by antitoxin.

TABLE 5.

COMBINING PROPERTIES OF TOXIN AND OF MODIFIED TOXIN FOR DIPHTHERIA ANTITOXIN (GLENNY, HOPKINS AND POPE)*

	Combining time with antitoxin: Four hours at room temperature				
1. Toxin Not Modified:					
Number of units of antitoxin added to 1 cc. of toxin..	15	20	30	40	50
Number of units detected after four hours............	5	8	18	28	38
Number of units combined	10	12	12	12	12
Total combining power, 12 units per cc.					
	Four hours at room temperature				
2. Toxin Modified:					
Number of units of antitoxin added to 1 cc. of toxin..	15	20	30	40	50
Number of units detected after four hours............	10	14	22	31	42
Number of units combined	5	6	8	9	8
	Forty-eight hours at room temperature				
3. Toxin Modified:					
Number of units of antitoxin added to 1 cc. of toxin..	15	20	30	40	50
Number of units detected after forty-eight hours......	6	11	20	29	39
Number of units combined	9	9	10	11	11

* Lo dose of toxin, 0.18 cc. One cc. toxin neutralized by 5.5 units of antitoxin. Varying amounts of antitoxin in excess of 5.5 units added and after standing residual antitoxin titrated.

In the absence of the simpler animal test for the combining power of modified toxin, Glenny, Hopkins and Pope suggest the use of the flocculation test of Ramon. By this method it is easy to detect whether the combining power of toxin has altered during modification. Glenny and Okell have recently proposed the use of the symbol Lf comparable with Lr, Lo, for the amount of toxin equivalent to 1 unit of antitoxin, as determined by flocculation.

Table 6 shows the effect of exposing a toxin to various concentrations of formaldehyde at a temperature of 37° C. for twenty-four hours. The floccu-

TABLE 6.

SHOWING THE EFFECT ON A BATCH OF DIFFERENT TOXINS OF TREATMENT WITH VARYING CONCENTRATIONS OF FORMALDEHYD AT A TEMPERATURE OF 37 C. FOR TWENTY-FOUR HOURS (GLENNY, HOPKINS AND POPE)

Key Number	Percentage formaldehyde added. per cent.	Number of minimal reacting doses per cc.	Minimal lethal dose cc.	Lf. C.c.
J. 3488	Nil	500,000	0.002	0.11
Px 146	0.01	260,000	0.003	0.11
Px 147	0.03	150,000	0.01	0.11
Px 148	0.1	25,000	0.02	0.11
Px 149	0.2	5,000	0.05	0.12
Px 150	0.3	1,200	0.1	0.12
Px 151	0.4	50	2.0	0.12
Px 152	0.5	0	Over 5.0	0.12
Px 153	1.0	0	Over 5.0	0.12

lating doses recorded in the last column show that the total combining power of the toxin had not been greatly reduced, but that some loss had occurred. This could indicate that during the process of conversion of toxin into toxoid either destruction has occurred, or the affinity of the toxoid for antitoxin had been so greatly reduced that even the flocculating value had been affected. The latter is most probably true.

PERSONAL OBSERVATIONS

Several toxoid preparations were made by Banzhaf at the research laboratory according to the English method. These preparations of toxoid were used by Zingher for active immunization on an extensive scale among the school children of New York City. Tables 7 and 8 indicate in a striking way the results noted by him with two different preparations of toxoid.

(a) PREPARATION OF TOXOID USED FOR IMMUNIZATION

To each 1,000 cc. of toxin 1 cc. of formaldehyd was added. The bottles were shaken up and placed in the incubator, where they were allowed to remain undisturbed at a temperature of 38 C. for from four to five weeks. The preparations were then removed and tested for the minimum lethal dosage and the L +.

(*a*) Toxoid 437 used in the schools, enumerated in Table 7, was made from an old toxin which originally, two years ago, had a minimum lethal dosage of 0.001 cc. Before the addition of formaldehyd the toxin had a minimum lethal dosage of 0.01 cc. and an L + of 0.32 cc. After the addition of 0.1 per cent. formaldehyde and after remaining in the thermo tat for five weeks, the modified toxin was removed, and kept in the icebox until it was standardized and used for immunization.

The minimum lethal dosage of the toxoid was found to be 1.5 cc. (1 cc. killed a guinea-pig in thirteen days) and the L + 3.5 cc. The toxin had been reduced in toxicity by the action of formaldehyd and exposure to incubator temperature to the extent that 150 times the original amount of toxin represented one minimum lethal dosage. The combining power of the toxoid for antitoxin, however, had diminished much less, about ten times the amount of the original L + being represented by the new L +. The toxoid was diluted with salt solution, 1:20, 1:14 and 1:10, representing 5, 7.5 and 10 per cent. of the preparation.

The amino-acid nitrogen content of this toxoid, as determined by the van Slyke method, was 4.5 mg. for each 10 cc.

(*b*) Toxoid 377 was prepared from another toxin, which had a minimum lethal dosage of 0.01 cc. and an L + of 0.27 cc. One liter of the formaldehyd treated toxin, which had been kept in the incubator for four weeks and one liter which had been kept for five weeks, were mixed together and standardized. The new minimum lethal dose was approximately 1.0 cc. (1.0 cc. killed a guinea-pig in 4.5 days) and the L + was 5.0 cc. In contrast with Toxoid 437 this preparation had not lost as much in toxicity, yet in combining power as represented by the L + dose it was only about one-half as strong. The animo-acid nitrogen content of this toxoid was 5.1 mg. per 10 cc. These differences probably explain the difference in immunizing value noted between the two preparations.

(*b*) LOCAL AND CONSTITUTIONAL REACTIONS AFTER INJECTIONS OF TOXOID

The reactions following the injections of toxoid were rather mild when compared with those noted after injections of the old type 3 to 5 L + mixtures of toxin-antitoxin. The protein effect of the diluted toxoid was slightly more marked than that of the 0.1 L + mixtures of toxin-antitoxin. This was due to the dilution of the toxoid being from ten fold to twenty fold as compared with the thirty fold dilution of the toxin in the 0.1 L + toxin-antitoxin. The local toxic effect, however, of the diluted toxoid was decidedly less pronounced than after the 0.1 L + mixtures. This was due to the fact that each dose of toxoid represented only from one-twentieth to one-thirtieth of a minimum lethal dosage, as compared with the one-eighth minimum lethal dosage in each dose of the toxin-antitoxin.

(*c*) IMMUNITY RESULTS WITH TOXOID

Table 7 shows the results noted with Toxoid 437. It was diluted to 5 per cent. (1:20) for Group A, to 7.5 per cent. (1:14) for Group B and to 10 per cent. (1:10) for Group C. The table shows the results with two doses and with three doses of 1 c.c. each, given at intervals of from one to two weeks. Over 1,800 injected children were retested.

TABLE 7.

RESULTS OF ACTIVE IMMUNIZATION WITH DIPHTHERIA TOXOID (MODIFIED TOXIN) (ZINGHER)

Preparation No. 437. Tests by Banzhaf
Minimum lethal dosage of toxin two years ago, 0.001 c.c.
Before modification with 0.1 per cent. formaldehyde, minimum lethal dosage 0.01 c.c. L + 0.32 c.c.
After modification with 0.1 per cent, formaldehyde and after five weeks in thermostat, minimum lethal dosage 1.55 c.c. (150×) (Weaker). L + 3.5 c.c. (10×) (Weaker)
Nitrogen content by van Slyke method, 4.5 mg. per 10 c.c.

		Original Schick test		Diluted toxoid 1 cc.				Two doses toxoid			Three doses toxoid		
	Public School	Number tested	Per cent. positive	Dilution	Per cent. in dilution	Mos. after injection	Total re-tested	Number	Schick negative	Per cent. negative	Number	Schick negative	Per cent. negative
A.	103 M.*...	471	37.1	1:20	5.0	3	136	24	12	50.0	112	94	84.0
B.	14 M.....	686	18.5	1:14	7.5	3	125	25	20	80.0	100	98	98.0
	19 M.....	841	22.0	1:14	7.5	3	142	20	15	75.0	122	120	98.3
	83 M.....	625	17.3	1:14	7.5	3	81	14	14	100.0	67	66	98.5
	Total....	2,152	....	....	..	..	484	83	61	75.2	401	378	94.2
C.	15 M.....	714	25.6	1:10	10.0	3	145	11	20	55.0	134	141	95.0
	23 Bx.*...	600	24.8	1:10	10.0	3	98	15	11	73.3	83	77	92.7
	11 Bx.....	205	69.2	1:10	10.0	3	118	51	35	68.6	67	62	92.5
	32 Bx.....	174	30.0	1:10	10.0	3	29	3	2	66.6	26	24	92.3
	20 M.....	713	15.0	1:10	10.0	3	106	20	12	60.0	86	79	91.8
	3 Bx.....	741	24.4	1:10	10.0	3	132	27	21	77.7	105	94	90.4
	68 M.....	642	49.5	1:10	10.0	3	210	48	24	50.0	162	133	83.0
	85 M.....	725	11.1	1:10	10.0	3	43	16	12	75.0	27	24	81.5
	54 M.....	349	51.7	1:10	10.0	3	102	21	12	57.1	81	60	74.0
	61 M.....	1,253	25.5	1:10	10.0	3	274	33	18	54.5	241	176	73.0
	Total..	6,116	....	....	....	.	1,257	245	167	68.1	1,012	870	86.0

* M., Manhattan; Bx., Bronx.

Groups A and B have been added together. With two doses of the toxoid, sixty-one of eighty-three children, or 75.2 per cent., became immune;

with three doses, 378 of 401 children, or 94.2 per cent., were successfully immunized. In Group C the toxoid, although less diluted, gave somewhat poorer results. Of 245 children receiving two doses, 167 or 68.1 per cent., became immune and gave a negative Schick retest; of 1,012 children receiving three doses 870, or 86.0 per cent., were immunized and showd a negative reaction at the retest.

The results with this preparation of toxoid compare quite favorably with the immunity results formerly noted with many mixtures of toxin-antitoxin. As shown by Zingher in judging the immunizing value of a preparation of toxin-antitoxin, it is necessary to consider other factors besides the mixture itself. One of the most important of these is the previous exposure and slight immunization by contact of the injected children. It was found that the children of the poorer classes, showing a low percentage of positive reactions at the original test, when injected with the same preparation of toxin-antitoxin, gave a better antitoxin response than those of the more well-to-do, who had been kept fairly segregated and had had only slight exposure to repeated contact infection with the diphtheria bacillus. To a certain extent the same factors were found to hold true in the immunity results noted after injections of toxoid.

Table 8 gives the results with Toxoid 377. The immunity response was much poorer than with Toxoid 437. The dilution of the toxoid used in these schools was 5 per cent. (1:20). In other schools we injected the toxoid in a dilution of 7.5 per cent. (1:14), and these schools will be retested within a few months. With three doses of the 1:20 toxoid dilution, only 114 out of 245, or 46.5 per cent., were successfully immunized. The explanation given, that the actual combining value of this toxoid preparation was less than that of Toxoid 437, even though its toxicity for the guinea-pig was greater, probably explains these results.

TABLE 8.

RESULTS OF ACTIVE IMMUNIZATION WITH DIPHTHERIA TOXOID (MODIFIED TOXIN) (ZINGHER)

Preparation No. 377
Minimum lethal dosage of toxin two years ago, 0.001 c.c.
Before modification with 0.1 per cent. formaldehyd, minimum lethal dosage 0.01 c.c. L + 0.27 c.c.
After modification with 0.1 per cent. formaldehyd after five weeks in thermostat, minimum lethal dosage 1.00 c.c. (100×) (weaker). L + 5.0 c.c. (18.5 ×) (weaker)
Nitrogen content by van Slyke method, 5.1 mg. per 10 c.c.

	Original Schick Test					Two Doses Toxoid			Toxoid Three Doses		
Public School	Number tested	Per cent. positive	Toxoid dilution	Mos. after injection	Total retested	Number	Schick negative	Per cent. negative	Number	Schick negative	Per cent. negative
105 Manhattan........	296	32.0	1:20	3	71	00	00	00.0	71	36	50.7
51 Manhattan........	399	21.0	1:20	3	88	13	10	76.9	75	44	58.6
84 Manhattan........	330	31.2	1:20	3	61	19	6	31.5	42	21	50.0
37 Bronx..........	357	26.3	1:20	3	61	4	2	50.0	57	16	27.4
Total............	1,382	....	....	..	281	36	18	50.0	245	114	46.5

The retests in the schools shown in Tables 5 and 6 were given from 2.5 to 3 months after the injections. Zingher has found in his work with toxin-antitoxin that a second Schick test made a year later on the positive reactors will show that more than 50 per cent. will have become immune without their having received further injections of toxin-antitoxin. It is interesting to note in this connection that many of the children who are shown in Tables 7 and 8 as persistent positive Schick reactors, gave a much weaker reaction than in the original test. It is probable, therefore, that many will show a negative Schick reaction at a later retest.

COMMENT

The value of toxoid as an immunizing agent against diphtheria lies in its easy and simple preparation and standardization. It is also a very safe preparation to use.

The possibility that persons become sensitized to horse serum by the injections of toxin-antitoxin was indicated by the intradermal tests reported by Hooker. A repetition of Hooker's investigations by one of us gave somewhat different results, in that while those injected with toxin-antitoxin showed on retest an increased percentage of skin reactions to horse serum, yet this was much less than Hooker found. There is also great doubt as to the importance of the slight amount of sensitization. The knowledge of an immunization should not cause us to withhold antitoxin as persons have been repeatedly injected with antitoxin after previous injections of toxin-antitoxin, and no greater amount of clinical symptoms of sensitization was noted than after injections in persons who had not previously received toxin-antitoxin. However, to obviate even the slight possibility of danger, goat antitoxin is being substituted now for horse antitoxin in the preparation of toxin-antitoxin.

An observation made by Zingher in connection with the Schick retests after immunization with one-tenth L + mixtures of toxin-antitoxin is significant in connection with the question of sensitization after injections of these mixtures. He found that not only were from 80 to 95 per cent. of the injected children immunized, but also that over 50 per cent. of these children showed slight to marked pseudo-reactions in the Schick retest. With the 3 to 5 L + mixtures such a high percentage of pseudoreactions was never noted at the retests. The question comes up whether very small amounts of proteins contained in the one-tenth L + mixtures are not apt to be sensitized to a somewhat more intense degree than the larger amounts represented by the 3 to 5 L + mixture. Whatever the explanation may be, this sensitization after one-tenth L + mixtures has no clinical significance except so far as indicating the necessity for the sake of accuracy for always making a control test when the Schick retest is made to check up on the immunity results.

More important than the question of sensitization to horse serum after toxin-antitoxin injections, is the recent experience of White that a preparation of 1 L + of toxin-antitoxin after freezing became toxic, which has added further interest to the possibility of substitution of toxoid for toxin-antitoxin. Even though further investigation showed that the 0.1 L + preparation never became more toxic, yet a certain opposition, unreasonable though it may be, has developed among a portion of the lay population against the use of toxin-antitoxin which will be eliminated by the substitution of toxoid.

Further work no doubt remains to be done in perfecting toxoid as an immunizing agent against diphtheria. Similar work had to be carried out with toxin-antitoxin. But the results noted in this communication are sufficiently encouraging to continue the work on an increasing scale and to aim for a more perfect preparation. There is no immediate urgency to substitute toxin-antitoxin by toxoid. If, however, as is probable, a toxoid can be elaborated that will serve as a good immunizing agent, the time will not be far distant when such substitution will be found of advantage.

CONCLUSIONS

1. Both the new toxin-antitoxin and the toxoid preparations give good immunizing results with but slight local and constitutional reactions.

2. The toxoid has advantages in that there is no horse serum in it to cause sensitization, which happens to a slight degree with toxin-antitoxin. The substitution of goat antitoxin for horse antitoxin will obviate this difficulty and this change is now being made.

3. Such a toxoid would do away with the fear in the population created by the recent experience with frozen toxin-antitoxin.

4. A satisfactory toxoid preparation is probably easier to make and also more stable.

5. In making toxoid by the addition of formaldehyde to toxin, care has to be taken of the amount of formaldehyd added and the period of time the formaldehyd treated toxin is exposed to the temperature of the incubator. The amount of amino-acid nitrogen, as determined by the van Slyke method, has a definite bearing on the amount of formaldehyd that is necessary to be added. Many of the toxin preparations containing from 2.5 to 5 mg. of nitrogen per 10 c.c. will require the addition of 0.1 per cent. formaldehyde and the exposure in the incubator from four to five weeks.

6. Careful tests of the minimum lethal dosage, the L + and the flocculating value of the toxoid should be made in standardizing the preparation. The minimum lethal dosage represents the remaining trace of free acting toxin, the L + and the flocculating value, the combining power of the toxoid. The combining value of toxoid serves as a good index of its immunizing value. It is of advantage to have a slight excess of free toxin (from 1/15 to 1/20 minimum lethal dosage in each dose) in the toxoid.

7. Toxoid and toxin-antitoxin should be carefully studied and, if possible, improved, and the one that is shown to be the best adopted for general use.

For bibliography see the original article.

THE NEW MIXTURE (1/10 L +) OF TOXIN ANTITOXIN

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William H. Park, E. J. Banzhaf and Abraham Zingher

The extensive work in active immunization with diphtheria toxin-antitoxin, which was carried on during the past three years in the schools and baby health stations of this City, has enabled us to make a number of interesting observations and has led to certain conclusions as to the type of mixture of toxin-antitoxin that would have to be used before we could hope for a wide acceptance of this form of protection against diphtheria.

Until a short time ago we had used quite extensively mixtures of toxin-antitoxin that contained from 3 to 6 L + doses of toxin per cc. These mixtures were prepared from strong toxins which were only slightly diluted before the addition of the antitoxin. They contained relatively large amounts of different proteins which gave rise to the disagreeable local and constitutional symptoms associated with toxin-antitoxin immunization. Chief among these reacting substances is the autolyzed protein of the diphtheria bacillus, which is contained in the diphtheria toxin broth culture.

One of the important questions that had to be decided was whether a mixture of toxin-antitoxin with a large number of L + doses per cc. would give better immunity results than one with a smaller number of L + doses. We therefore used different mixtures in the schools, containing between 3 and 6 L + doses per cc. *We found that mixtures with the larger number of L + doses were not more efficient than those with the smaller number of L + doses, as long as the mixtures were equally toxic* (*underneutralized*). In fact we noted that mixtures with the *smaller* number of L + doses per cc. but showing greater toxicity for the guinea-pig, gave *better immunity results* than those with the larger number which were more neutralized.

On the basis of these data Dr. Park thought that much smaller amounts of toxin might be equally valuable and asked Dr. Banzhaf to prepare mixtures of toxin-antitoxin which contained only a fraction of an L + dose of toxin per cc. These mixtures were to be balanced, however, in such a way (see Fig. 1) that the toxic or underneutralized fraction of the toxin-antitoxin was similar to that of mixtures containing from 3 to 6 L + doses of toxin per cc., which had been found to give an active immunity in a large percentage of children. By diluting the toxin and thus greatly diminishing the reacting protein content of the mixture, it was hoped to eliminate one of the most objectionable features of diphtheria toxin-antitoxin immunization. Fig. 1 shows that in the new type mixture, 29/30 of the neutralized fraction of toxin-antitoxin has been eliminated by dilution, while the free* or toxic fraction has been retained in the same proportion as in an efficient old type (3 L +) mixture.

PREPARATION OF THE NEW MIXTURE

Several mixtures of toxin-antitoxin, containing from 1/20 to 1/2 L + doses of toxin per cc. were prepared, some of these being slightly more toxic than others. After these mixtures had been used in a number of institutions by Schroeder a standard mixture was adopted with a definite fraction of an

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*The words "free toxin" are used in this article to indicate the proportion of toxin in a mixture of toxin-antitoxin which is not neutralized by antitoxin.

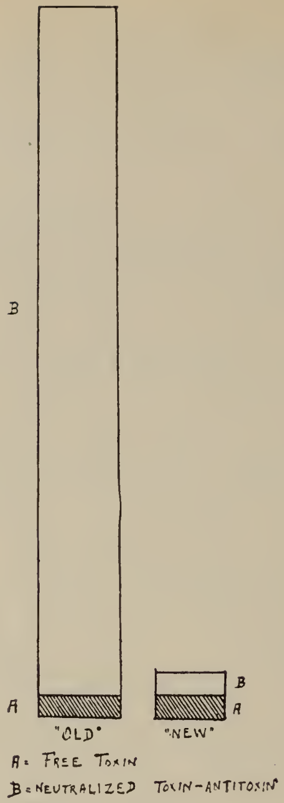

Fig. 1

Diagram of the composition of "old" and "new" toxin-antitoxin mixtures, each containing the same amount of free toxin. "Old" contains 3 L+ doses of toxin and 3.5 units of antitoxin per cc. "New" contains 0.1 L+ dose of toxin and 0.075 unit of antitoxin per cc. (Reprinted by courtesy of N. Y. State Journal of Medicine.)

L + dose of toxin per cc. This standard mixture was taken to be one containing 1/10 L + per cc. and representing about 4 M. L. D. of our toxin.

Park, Schroeder and Zingher reported in the *American Journal of Public Health* (January, 1923), from observations made by Dr. Schroeder in a few schools, that 90 per cent of 490 children became immune after three doses of a 1/10 L + mixture of toxin-antitoxin. Of 318 children injected with a 3 L + mixture, 92 per cent. became immune. Marked local reactions were noted in 22 per cent. with the old mixture and in none with the new type mixture.

The new mixture of toxin-antitoxin containing 1/10 L + per cc. is prepared by Dr. Banzhaf as follows: The antitoxin is added to the toxin in the proportion of 0.75 unit of antitoxin to one L + dose of toxin, the mixture allowed to stand for three to four hours and then diluted, so that each cc. represents 1/10 L + dose of toxin. The diluted mixture is subsequently passed through a Berkefeld filter, a sample of the mixture being removed before and after filtration to determine its toxicity in the guinea-pig. Six guinea-pigs are injected with each sample, two receiving 1 cc. each, two 3.0 cc. and two 5.0 cc. Daily observations are made on the loss of weight, local induration at the site of injection and late paralysis. If the mixture is found to be too toxic it is allowed to remain at ice-box temperature for one to three months. It is then tested in a similar way until the desired toxicity is found to be present. The preservative added to the mixture of toxin-antitoxin is 0.5% phenol. We have found that tricresol added as a preservative to toxin-antitoxin is too irritating locally, the children complaining of a severe burning sensation at the time of the injection.

STANDARD OF TOXICITY OF THE NEW MIXTURES

As a result of rapidly accumulating experience with mixtures of toxin-antitoxin of different degrees of toxicity, we have come to the conclusion that an *underneutralized mixture, of which* 5.0 *cc. causes acute death of the guinea-pig in five to six days,* 3.0 *cc. death in six to ten days, and* 1.0 *cc. paralysis in fifteen to eighteen days, and death in eighteen to twenty-five days is the best type of mixture to be used for active immunization.*

LOCAL AND CONSTITUTIONAL REACTIONS PRODUCED BY NEW AND OLD TYPE MIXTURES

The local and constitutional reactions obtained with mixtures of toxin-antitoxin are due to two different substances in the mixtures:

(A) In the old type mixtures, containing from 3-6 L + doses of toxin per cc., there was a considerable amount of autolyzed bacillary substance and other kinds of protein derived from the broth culture itself, which gave rise in sensitive individuals to the most marked forms of local and constitutional reactions. These reactions were of an anaphylactic type and generally occurred in hypersensitive individuals, who had given a negative-pseudo or positive-combined reaction. Both the local and constitutional reactions reached their height within twenty-four hours, gradually disappeared in two to three days, although they persisted at times for four to six days. The local reaction varied from an area of redness and induration the size of a 50-cent-piece to an extensive infiltration involving one-half or more of the arm. There was pain, tenderness and slight glandular enlargement in the axilla. Fever was present, the temperature ranging from 100-104° F. A few of the individuals showing simple positive Schick reactions also had slight local and constitutional disturbances, but the more marked reactions to the toxin-antitoxin were found only in those who had shown themselves to be hypersensitive to the proteins of the mixture by their negative-pseudo or positive-combined reactions.

(B) With the new type of mixture (1/10 L +) the local and constitutional effects due to the reacting protein were to a large extent eliminated, but the local effect of the larger amount of free toxin present in some of these preparations became more evident. We noticed, following the subcutaneous injections, especially with mixtures of which 1.0 cc. caused death of the guinea-pig in six to eight days and 3.0 cc. in three to five days, local reactions that were slow in appearing, but much more lasting than the protein reactions. These reactions were characterized by local induration and redness, which gradually faded into a brownish-red area of discoloration. The area had a dry, wrinkled, desquamating appearance and resembled closely on inspection a large positive Schick reaction. With one of these mixtures we even noticed in a few children a superficial necrosis of the skin. This local reaction was due to a large extent to the free toxic fraction of the mixture.

The majority of the mixtures of toxin-antitoxin used in the schools were less toxic and corresponded more to the degree of underneutralization given for a standard preparation. With such mixtures the local reactions were only slight and often absent. It was possible, in fact, to inject several hundred school children with these new type mixtures during a day's work without hearing subsequently of complaints about sore arms, or the necessity of the children remaining away from school for a day or two. Constitutional symptoms like fever, were practically absent. This was noticeable even after injecting the more toxic preparations, and stood out in striking contrast to the disagreeable effects seen with the 3-6 L + mixtures in 5-20 per cent. of school children.

Limits of Underneutralization

The rather severe local effects of some of the more toxic mixtures of toxin-antitoxin indicate the upper limit of toxicity within which we have to confine ourselves if we wish to avoid disturbing local reactions. If we take care, however, not to exceed this toxicity in the new mixtures and adhere more or less closely to the degree of underneutralization indicated as a suitable standard, we shall find in the 1/10 L + mixture of toxin-antitoxin an ideal preparation for the active immunization against diphtheria.

Stability of the New Mixture

Recent results obtained with the old type mixtures (3-5 L + per cc.) of toxin-antitoxin (Table 1) indicate that these mixtures do not lose to any extent their *immunizing* value even after standing for several months. Some of these mixtures had lost nearly all their *toxicity* on standing and appeared almost neutralized in the guinea-pig test. The slight excess of free toxin being changed into toxoid, *these favorable immunity results indicate that toxoid can give rise to antitoxin antibodies and that it is probably an important factor in active immunization.* We are giving three injections of diluted diphtheria toxoid at the present time to determine its value in producing an active immunity against diphtheria. A similar method has been suggested by Glenny and Hopkins.

TABLE 1.

RESULTS OBTAINED WITH FRESH MIXTURES AS COMPARED WITH OLDER MIXTURES OF TOXIN-ANTITOXIN CONTAINING FROM 3 TO 5 L + TOXIN PER CC.

Mixture	No. L+ in each CC.	No. M. L. D. in each CC.	Amount injected	Action on Guinea-Pig	Results in Children			
				Result	No. Doses T-A	Total No. Schick Retested	Schick Negative	Per Cent. Negative
44 Fresh	3	120	1.0 cc. 5.0 cc.	Acute death in 6 days. Acute death in 3 days.	3	1,080	1,047	97.0
44* Old	3	120	1.0 cc. 3.0 cc. 5.0 cc.	Slight local effect, death from pneumonia. Moderate local effect, death in 12 days. Moderate local effect, death in 9 days.	3	142	137	96.4
42 Fresh	5	200	0.5 cc. 1.0 cc. 5.0 cc.	Paralysis, death in 17-20 days. Paralysis, death in 17 days. Acute death in 3 days.	3	3,846	3,356	87.2
42* Old	5	200	1.0 cc. 2.0 cc. 5.0 cc.	No local effect, no paralysis, recovery. No local effect, no paralysis, recovery. Slight local effect.	3	364	361	99.1

*The mixtures were kept at ice box temperature for 7 months.

IMMUNOLOGICAL STUDIES ON TYPES OF DIPHTHERIA BACILLI

I. Agglutination Characteristics
II. Protective Value of the Standard Monovalent Antitoxin

Published in The Journal of Immunology, 1922, Vol. VII, pp. 243—252

William H. Park, Anna W. Williams and Alice G. Mann

The subject of this communication would have been exhaustively investigated long ago if there had been practical evidence that a monovalent diphtheria antitoxin did not protect from diphtheria. It is true that in regard to *B. tetani* and *B. botulinus* laboratory investigations have given contradictory results as to the efficacy of a monovalent serum. These investigations have shown us: first, that tetanus bacilli, although they are divided into several types by their response to agglutinins, nevertheless, all produce toxins which are neutralized by a monovalent antitoxin; second, that the reverse is true of the bacilli causing botulism, in so far as the production of toxin is concerned. Among the strains of *B. botulinus* there are at least two different types producing toxins which respond only to homologous antitoxins.

As to diphtheria bacilli, however, there are two series of observations which have been made concerning the degree of protection afforded by monovalent diphtheria antitoxin. These observations have seemed to give all-sufficient evidence that the toxins produced by the different strains of the diphtheria bacilli are alike, at least, in their affinity for antitoxin. The first is, that for the last twenty-five years, ever since diphtheria antitoxin has been used in practice, injections of a monovalent antitoxin in hundreds of thousands of persons known to be in contact with infection, have given practically complete protection for two weeks—the period during which antitoxin is known to remain in appreciable amounts in a human being after injection. The second series of observations is that routine virulence tests throughout the world have with few, if any, exceptions shown that a monovalent diphtheria antitoxin is able to protect animals against a dose of diphtheria culture fatal to other animals not given antitoxin.

If any doubt has existed in regard to the universal worth of a monovalent diphtheria antitoxin, it is one that has led to experimental investigation from time to time as to whether an antitoxic serum alone was as efficient as a combined antibacterial and antitoxic serum and whether from this standpoint a polyvalent serum might not be more advantageous. But the paper published by Havens in 1920 seemed to show that diphtheria bacilli were divided into two agglutinative groups and that each group produced a toxin that was quantitatively at least, a very different one from that produced by the other

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This article combines the results of portions of two investigations. The first portion was a part of our studies on the respiratory infections and was designed to show the relative values of the absorption-agglutination method and other serological tests in determining epidemic strains. The second portion was a part of a series of studies on the similarity of the toxins prodouced by the diphtheria bacilli and the adequacy of a monovalent antitoxin.

In the studies on the absorption-agglutination characteristics of the diphtheria bacilli, we were given valuable aid by technicians supplied by the Influenza Commission of the Metropolitan Life Insurance Company.

group, so that a monovalent serum for group I had no appreciable effect on animals injected with group II toxin unless the serum was given in enormous amounts. These findings, if correct, are of so much practical importance that reference will be made to them in some detail in connection with our own work.

I. Agglutinative Types of B. Diphtheria

A number of investigators have reported the obtaining of an agglutinating serum that differentiated between "true" and "pseudo" diphtheria bacilli (Schworer, Sander, Mason, but no one had described definite agglutinative types among the toxic strains until Durand published his observations in 1918. Durand announced that he had obtained four agglutinative types. In 1920 he published a further report in which he stated that on examining 252 strains by this test he found that they were divided as follows:

Type A contained	16	strains
Type B contained	8	strains
Type C contained	31	strains
Type D contained	76	strains
Type E contained	51	strains
Unclassified	71	strains

These types were strictly specific in that each absorbed only the respective agglutinins from the homologous serum.

Early in 1920 Dr. Durand visited our laboratory, demonstrated his methods, presented us with his type strains which he now designated with figures instead of letters and gave us the following facts in regard to their sugar fermentations:

	MALTOSE	DEXTRIN	GLYCEROL	GALACTOSE	SACCHAROSE
Type I—Americaine ("our No. 8")	+	+	–	–	–
Type II—Durand	–	–	–	–	–
Type III—Nodet	+	+	+	+	–
Type IV—Benjamin	+	+	+	+	–
Type V—Sirbeaux	+	+	+	+	–

All ferment dextrose and levulose.

Dr. Durand also gave figures to support the following observation: There was less paralysis and attendant mortality in cases of diphtheria treated with diphtheria serum produced by the inoculation of both toxin and bacilli; i.e., an antitoxic and "antibacterial" serum, than with serum produced by the use of toxin alone. As Durand used all of the type strains in making this bacterial serum he had no evidence as to the effect of a monovalent antibacterial serum.

Our own work on agglutination. We immediately began inoculating a series of horses with Durand's type strains and with our own strain of "No. 8," as well as with several strains from each of two apparent epidemics of diphtheria, one in an institution in New York City and the other in a village near the City.

The horses were inoculated intravenously every day for three day periods, with suspensions of cultures prepared as follows:

The bacteria were grown for eighteen hours on Loeffler's coagulated blood serum medium at 37° C. The growths were then scraped into 0.85 per cent. salt solution and transferred to tared Purdy tubes. They were sedimented in the centrifuge for twenty minutes and the supernatant liquid was removed. The weight of moist sediment was determined and the sediment was suspended in 10 cc. salt solution. The required weight of sediment (in suspension) was measured off and made up to 15 cc. for injection.

TABLE 1.

DIRECT AGGLUTINATION OF THE STRAINS OF B. DIPHTHERIAE USED FOR TOXIN-ANTITOXIN COMPARISON

STRAINS	HORSE SERUM TITER					
	No. 8	Nodet	Sirbeaux	Benjamin	11	3362
Type I. Our "no. 8"	1600	0	0	0	0	
Type I. Americaine	1600	0	0	0	0	
Type III. Nodet	0	1600	0	0	0	0
Type IV. Benjamin	0	0	0	800	800	
Type IV. 11	0	0	0	0	0	
Type V. Sirbeaux	0	0	400	0	0	
No. 3362	0	0	0	0	0	400

The first dose was 1 mgm.; each succeeding dose was doubled unless the condition of the horse contra-indicated it. After each three day period, the horse was allowed to rest for seven days. The first three doses of the bacillus emulsion were heated at 55° C. for one hour before inoculation. Later, living cultures were inoculated. From four to six series of inoculations were given each horse.

In table 1 are given only the strains which were used by us to test the protective action of our monovalent serum.

With the serums obtained, we corroborated fully the work of Durand showing that there are at least five agglutinative types of diphtheria bacilli.

Havens kindly sent us four strains of his group II and we found that none of them agglutinated with our "No. 8" serum.

Havens did not mention Durand's work in his article. He reported the results of his own study of 206 strains. These were from acute cases, convalescents and carriers. All varieties of morphologic cultures were tested but he says that "morphology bears no observed relation to agglutinating properties." A certain strain was chosen to inoculate rabbits for the production of agglutinating serum. Havens does not give the dosage or the course of injections. He simply states that the injections were made intravenously in increasing amounts, and that an agglutinating serum was obtained with a titer of 4860 for the homologous strain. Of the series of 206 strains, 169 agglutinated in this serum to the same titer as the homologous strain, while 37 failed to agglutinate in any dilution. One strain of this second group was chosen to inoculate a rabbit for the production of agglutinating serum. This serum gave a titer of 4800 or higher. The other 36 strains of this group agglutinated to the same titer. He concludes as follows:

Judged by the evidence furnished by the agglutination test there are two biologic groups of the diphtheria bacillus. No evidence of cross agglutination was found among the members of this series [obtained in Iowa City].

All members of the series fell into one of the two groups with no differences observed in the degree of agglutinability. As judged by the results of agglutination, two groups exist which include all strains of the diphtheria bacillus.

Havens said that the virulence in animals was approximately the same in the two groups.

When we consider the results of Havens in connection with Durand's and those of our own, it seems fair to infer that in the comparatively small city from which he drew his material, only two types of bacilli were present.

There can be no doubt whatsoever that in all large communities there are many types and at least several dominant ones.

II. Protective Action of Diphtheria Antitoxin (Standard No. 8), Against Different Agglutinating Types of B. Diphtheriae

The question of the protection results is so important that we quote Havens' experiments in detail. In attempting to show "the protective properties of diphtheria antitoxin with reference to biologic groups," he reports first the following tests with various cultures and diphtheria antitoxin No. 8.

HAVENS' TESTS

CULTURE	GROUP	AMOUNT	UNITS ANTITOXIN	TYPE ANTITOXIN	RESULT
210	I	cc. 1	None		Died, 48 hours
210	I	1	100	I	Lived
221	II	1	None		Died, 36 hours
221	II	1	100	I	Died, 36 hours
141	II	1	None		Died, 48 hours
141	II	1	100	I	Died, 48 hours

The results of these tests of Havens indicate that group I antitoxin has no neutralizing effect upon the toxins produced in the animals by group II cultures. Similar results followed when he injected cultures intracutaneously. Six strains of group I bacilli were inoculated into guinea pigs that had been injected with 100 units of group I antitoxin. No cutaneous lesions were produced. Eighteen strains of group II bacilli were inoculated into guinea pigs that had received 100 units of group I antitoxin. The reactions in 15 were identical with the controls, while those in three were slightly less than the controls.

Before discussing Havens' results with toxin, we give in table 2 our findings with cultures of at least five different agglutinating types including three of Havens' "group II" strains. The cultures were heavy eighteen hour growths in veal broth.

TABLE 2

LIFE-SAVING EFFECTS OF TYPE I ANTITOXIN IN ANIMALS INJECTED WITH BACILLI OF VARIOUS TYPES

TYPE	WEIGHT OF GUINEA PIG	AMOUNT OF CULTURE	UNITS OF ANTITOXIN NO. 8	RESULT
	grams	*cc.*		
Type I. (Our no. 8, New York City)	248	2.0	None	Died 3 days
	230	3.0	None	Died 2 days
	355	3.0	None	Died 2 days
	230	3.0	0.2	Died 2 days
	230	3.0	0.5	Died 2 days
	360	3.0	1.0	Remained well
	355	3.0	2.0	Remained well
Type III. (Durand's Nodet, France)	225	0.2	None	Died 2 days
	225	0.2	0.5	Remained well
Type V. (Durand's Sirbeaux, France)	235	0.2	None	Died 9 days
	400	0.75	None	Died 4 days
	235	0.2	0.2	Died 8 days
	405	0.75	1.5	Remained well
Type IV. (No. 11, New York City)	242	2.0	None	Died 3 days
	245	2.0	0.5	Remained well
Type IV. (Durand's Benjamin, France)	240	0.05	None	Died 2 days
	235	0.1	0.2	Died 6 days
	235	0.05	0.05	Remained well
No. 3362, unclassified (New York City)	245	0.2	None	Died 3 days
	255	0.2	0.2	Died 4 days
	255	0.02	0.5	Remained well
B, unclassified (Near New York City)	235	0.1	None	Died 6 days
	245	0.1	0.2	Remained well
No. 151 (Havens' group II, Iowa City)	237	1.0	None	Died 3 days
	262	1.0	None	Died 2 days
	255	1.0	0.2	Remained well
	245	1.0	0.5	Remained well
No. 184 (Havens' group II, Iowa City)	225	0.2	None	Died 2 days
	232	0.2	None	Died 2 days
	245	0.2	0.2	Died 6 days
	230	0.2	0.5	Remained well
No. 8347 (Havens' group II, Iowa City)	217	0.1	None	Died 10 days
	440	0.3	None	Died 2 days
	235	0.3	0.5	Died 3 days
	225	0.3	1.0	Died 3 days
	215	0.3	5.0	Remained well
	255	0.3	5.0	Remained well

The results given in the table do not show any striking difference in the protecting value of the type I antitoxin, when used in animals inoculated with a type I strain or with strains of other types. All who have worked extensively with living cultures have discovered that the results are much more irregular than when toxins are used. The cultures vary from day to day, according to the culture medium used, in the abundance of their growth and in the vigor and apparent toxicity of the bacilli; a much larger number of units of antitoxin is required to protect against a fatal dose of living bacilli than against a fatal dose of toxin. There is nothing in our results to indicate to us that there is any marked difference in the pathogenic power of the different types of bacilli.

The last three cultures are of unusual interest since they were sent us by Havens as examples of his Group II strains. All produced toxin of moderate strength when grown in bouillon. It is to be noted that our antitoxin produced by type I No. 8 bacillus was capable of protecting the animal which had received a subcutaneous injection of an amount of culture which produced death in the control animal within two days. Indeed, the type I antitoxin appears to be as efficient against two of the cultures sent by Havens as against strain "No. 8." Although with the third culture a considerably larger quantity of antitoxin was required, it is noted below in table 3 that the toxin produced by this culture was neutralized by the usual amount of antitoxin.

The experiments of Havens showing the neutralizing results of mixtures of his group I antitoxin and group II toxin are given in the following tabulation:

HAVENS' TESTS

STRAIN NUMBER	TOXIN GROUP	MINIMAL LETHAL DOSES	GROUP I ANTITOXIN UNITS	RESULT
53	II	2	10	Died, 72 hours
53	II	2	—	Died, 72 hours
74	II	2	5	Died, 48 hours
74	II	2	20	Lived, marked virulence
74	II	2	25	Lived, oedema
74	II	2	50	Lived, no lesions
34	I	2	5	Remained well
34	I	2		Died in 48 hours

The group II toxin required far larger amounts of group I antitoxin to neutralize it than did the group I toxin.

NEUTRALIZING EXPERIMENTS WITH MIXTURES OF GROUP II ANTITOXIN AND GROUP I TOXIN

STRAIN NUMBER	TOXIN GROUP	MINIMAL LETHAL DOSES	GROUP II ANTITOXIN UNITS	RESULT
34	I	1	5	Died, 96 hours
34	I	1		Died, 96 hours

As seen in the above tabulation, one minimal lethal dose of group I toxin was not neutralized by five units of group II antitoxin.

The fact that in Havens' tests very large amounts of group I antitoxin usually neutralized 2 minimal lethal doses of group II toxin, indicates that the group I antitoxin contained the same neutralizing substances for group II toxin as for group I toxin, but that there were quantitative differences in the proportion of the different antitoxic substances.

TABLE 3.

TOXIN RESULTS

TOXIN		WEIGHT OF GUINEA PIG	NUMBER OF MINIMAL LETHAL DOSES	NO. 8 ANTITOXIN	RESULT
From culture	Type				
		grams			
No. 8	I	250	1.0	0.02	Lived
Durand	II	230-260	1.0	0.02	Lived
Nodet	III	230-260	1.5	0.02	Lived
Benjamin	IV	230-260	2.0	0.02	Lived
No. 11	IV	230-260	1.0	0.02	Lived
3362	Unclassified	230-260	2.0	0.02	Lived
151	Havens, group II	230-260	1.0	0.02	Lived
8347	Havens, group II	230-260	1.5	0.1	Lived
8347	Havens, group II	230-260	100.0	10.00	Lived

Havens stated "the results may throw some light on those cases of diphtheria which are not benefited except by large amounts of antitoxin." He alluded also to the death rate remaining at 10 per cent in cases of diphtheria. He suggested adding his group II toxin to horse injections, to the Schick test toxin and also to toxin-antitoxin mixtures.

Table 3 gives the results which we obtained with neutralizing mixtures of our own antitoxin No. 8 and the types of toxin indicated.

It is evident from this table that with the strains obtained from New York, from France and from Iowa no such difference has been discovered, as Havens believes he found. It would take repeated experiments and many animals to prove whether or not there is any minor difference either quantitatively or qualitatively between these toxins produced by the different agglutinative types.

CONCLUSIONS

The group of diphtheria bacilli contain strains belonging to several agglutinative types.

The toxins formed by these different types are, however, qualitatively alike and, from the practical standpoint, quantitatively so. Whether or not there are slight quantitative differences, further studies will be necessary to show.

Strong toxin from any diphtheria bacillus strain is suitable for the Schick test and for immunization of man or animal.

A monovalent antitoxic serum is suitable for protective and curative measures against all diphtheria strains.

For bibliography see the original article.

HUMAN HYPERSENSITIVENESS TO WHOLE HORSE SERUM OR SERUM GLOBULINS FOLLOWING DIPHTHERIA TOXIN-ANTITOXIN INJECTIONS—ITS IMPORTANCE.

Published in The Journal of Immunology, 1924, Vol. IX, pp. 17—24

William H. Park and May C. Schroeder

This preliminary communication is made at this time because of the interest aroused by Hooker's communication at the December meeting of the Society of American Bacteriologists.

He observed that a considerable percentage of adults who had some months previously received three injections of diphtheria toxin-antitoxin (3 L plus preparation) developed a skin sensitiveness to horse serum.

Two series of observations are here presented, one on children, the other on adults which confirm Hooker's conclusion, that a specific skin hypersensitiveness may be induced, in a moderate percentage of children and adults by the injection of the minute amount of horse serum globulins contained in the toxin-antitoxin injections. The results of tests on untreated persons and on those who had received larger amounts of serum globulins are also given as a comparison. As the antitoxin globulin solution is universally used to supply the antitoxin in the toxin-antitoxin, the globulin solution is used as the testing substances in most cases. Where the whole serum was used as a control the results were almost identical.

Tables 1 and 2 give the results in one series of tests on two groups of children, the individuals in one of which had and in one of which had not received, so far as is known, injections of toxin-antitoxin. It will be noted that about one-half of the children in each group were injected intracutaneously with 0.02 cc. of a 1:10 dilution of horse serum, the other half receiving

TABLE 1.

REACTIONS FROM INTRACUTANEOUS INJECTION OF HORSE SERUM OR HORSE SERUM GLOBULINS IN ONE HUNDRED AND SIXTEEN CHILDREN WHO HAD PREVIOUSLY RECEIVED THREE INJECTIONS OF TOXIN-ANTITOXIN. OBSERVATIONS MADE FIFTEEN TO TWENTY MINUTES AFTER THE INJECTIONS

MATERIAL INJECTED	AMOUNT	WHEAL AND AREOLA		WHEAL ONLY		NO REACTION	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
	cc.						
Solution of antoxic globulins, 1:10 dilution	0.02	17	23	33	44.5	24	32.5
Horse serum, 1:10 dilution	0.02	15	35.7	19	45.3	8	19
Total		32	27.5	52	45	32	27.5

TABLE 2.

CONTROL OBSERVATIONS ON NINETY CHILDREN WHO SO FAR AS KNOWN HAD NOT RECEIVED PREVIOUS INJECTIONS

MATERIAL INJECTED	AMOUNT	WHEAL AND AREOLA		WHEAL ONLY		NO REACTION	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
	cc.						
Globulin solution, 1:10 dilution	0.02	9	20	13	29	23	51
Normal horse serum, 1:10 dilution	0.02	1	2	22	49	22	49
Total		10	11	35	39	45	50

the same amount of antitoxic globulin solution. The total amount of globulin in the solution was about double that contained in the same quantity of unrefined serum. The children who had received toxin-antitoxin had been injected with a mixture containing 0.1 L plus dose of toxin. This contains but one thirtieth of the amount of serum globulins as compared with the older form of mixture which Hooker had employed. The amount of horse serum globulin in each dose (1 c.c.) of the new mixture is only about 0.0002 of that in 1 cc. of normal horse serum.

Tables 1 and 2 show that the children who had received three injections of toxin-antitoxin one year previously exhibited a much higher percentage of reactors than the untreated children. This is especially true of those who were tested by the horse serum injections. It is possible, but not probable, that one or two of the control children who reacted to the globulin had been given toxin-antitoxin before they entered the institution. While the persistence of the wheal probably indicates a trace of sensitiveness in the skin still it seems desirable to limit the term hypersensitiveness to those in which an areola develops.

TABLE 3.

OBSERVATIONS ON THIRTY ADULTS WHO HAD PREVIOUSLY RECEIVED THREE TOXIN-ANTITOXIN INJECTIONS OF A 3 L PLUS PREPARATION

MATERIAL INJECTED	AMOUNT	WHEAL AND AREOLA		WHEAL ONLY		NO REACTION	
		ber Num-	Per cent.	Number	Per cent.	Number	Per cent.
	cc.						
Solution of antitoxic globulins, 1:10 dilution	0.02	10	33	17	57	3	10

TABLE 4.

CONTROL OBSERVATIONS ON ONE HUNDRED AND EIGHTY-SEVEN ADULTS WHO HAD NOT RECEIVED INJECTIONS OF TOXIN-ANTITOXIN NOR AS FAR AS KNOWN OF ANY SERUM

MATERIAL INJECTED	AMOUNT	WHEAL AND AREOLA		WHEAL ONLY		NO REACTION	
		Number	Per cent.	Number	Per cent.	Number	Per cent.
Globulin solution, 1:10 dilution ...	cc. 0.02	36	19	103	55	48	26

The second series of observations was made upon adult inmates of an institution for the care of the insane. In this second series we are more certain of the fact that the control cases had never received toxin-antitoxin than we were in the first series. The results in the adults are similar to those in the children except that there was a higher percentage of reactors among the untreated persons. In no one of these cases was the areola more than 2 inches in diameter.

As an additional control a series of observations was made on sixteen children who, because of positive Schick tests, received on their admission to the Willard Parker Hospital prophylactic doses of 1500 to 3000 units of antitoxic globulin solution. The results of the intracutaneous injections of horse serum in these children are given in table 5.

TABLE 5.

THE FOLLOWING PATIENTS HAD POSITIVE SCHICK REACTIONS ON ADMISSION AND WERE GIVEN PROTECTIVE INOCULATIONS OF ANTITOXIC GLOBULINS AND TESTED FOR SENSITIVENESS TO HORSE SERUM AT VARYING INTERVALS AFTER THEIR PROPHYLACTIC DOSES OF ANTITOXIN

CASE NUMBER	AGE	UNITS ANTITOXIN	DATE OF ADMINISTRATION	DATE OF TEST	0.1 CC. OF 1:10 HORSE SERUM	0.02 CC. OF 1:10 HORSE SERUM
1	10	2000	11/28	12/19	Small areola	Small areola
2	7	1500	11/30	12/19	Small areola	Small areola
3	2	1500	12/5	12/19	No change	No change
4	3½	2000	12/20	1/15	No change	No change
5	6	1500	11/23	12/19	Negative	Negative
6	2	1500	11/28	12/19	No change	No change
7	6	3000	12/21	1/15	Areola	Areola (small)
8	8½	2000	11/22	12/19	Marked areola	Marked areola
9	2½	2000	11/16	12/23	Negative	Negative
10	6	2000	12/15	1/15	Marked areola	Marked areola
11	4	2000	11/20	12/23	Slight areola	Wheal size of dime, slight areola, no pseudopods
12	6	5000	11/10	12/23	Marked areola, irregular, no wheal	Marked areola, irregular, no wheal
13	14	2000	12/6	12/19	Areola	Slight areola
14	13	5000	11/19	1/14	No change	No change
15	8	2000	12/4	12/19	Marked areola	Moderately positive
16	16	2000	12/29	1/15	Areola	Areola

In this series 62.6 per cent. of the cases showed evidences of definite skin hypersensitiveness by the development of both a wheal and an areola. This contrasts with 27 per cent. and 33 per cent. in those who had been treated with toxin-antitoxin. The reactions in six were moderate. The areola in these cases ranged from 1 to 2 inches in diameter. In the other four the reaction was more intense. In one the areola extended nearly around the arm.

These observations on these three series of cases, although they are made on a comparatively small number of persons, indicate clearly that a moderate proportion of those who have been injected with toxin-antitoxin develop skin hypersensitiveness to horse serum globulins, but that an injection of 0.5 cc. of serum or a solution of serum globulins causes this change in a much greater proportion of persons and produces in them a greater effect.

It is fair to conclude, therefore, that Hooker is correct in his conclusion that toxin-antitoxin contains enough serum globulin to sensitize moderately a certain proportion of those receiving three injections. This leads up to the question as to whether such a hypersensitive state as shown by the skin reaction indicates a peril to the persons showing it if later serum injections become necessary.

Some ten years ago I felt it wise to give an intramuscular injection of 5000 units of antitoxin to each of thirty children who had received 1000 units or more of antitoxin about four weeks previously. The majority showed within a few minutes following an intracutaneous injection of 0.04 cc. of serum globulin solution a more or less extensive wheal and areola. Many of these developed within a few minutes a rise of temperature and a local or generalized rash. In the most extreme reactions the temperature rose five degrees but within four hours it had returned to normal. This child never seemed sick. None of the thirty children showed any symptoms that were in the slightest degree alarming.

The practice of the Health Department has been from the first introduction of antitoxin to give it to patients suffering from diphtheria without regard to the presence of the skin reaction. So long as there was no history of asthma or objective signs of status lymphaticus the serum injections were given without fear of the sensitizing effects of previous injections. The results of the injections in the thirty cases just mentioned confirmed us in our opinion that at least a subcutaneous or an intramuscular injection of whole serum or of antitoxin globulin solution could be given properly in those requiring it without being guided by the reaction of the test for skin hypersensitiveness. We have continued this practice up to the present time and have had no serious accidents.

The gradual introduction of the intravenous method of giving the antitoxin globulin solution in severe cases of diphtheria and of whole serum in cases of pneumonia have made us consider very carefully whether it was wise to disregard the cutaneous test in the treatment of these cases. Recently several interesting observations have been made which bear on this matter. A child, J.P., tested intracutaneously developed after fifteen minutes a slight wheal with a surrounding area of erythema. Twelve thousand units of antitoxin were given intravenously. The child promptly became cyanotic, sat up and fought for air. Simultaneously a large wheal and an enlarged areola with definite long pseudopods developed at the site of the previous intracutaneous injection. Ten minims of a 1:1000 solution of adrenalin were injected intramuscularly and the symptoms quickly subsided. There was no chill. The temperature rose two degrees during the next four hours and fell by lysis. The result in this case might seem to indicate that even a very moderate intracutaneous reaction should act as a warning but the next case throws doubt on this reasoning. A few days afterwards one of the staff was found to have diphtheria. When tested by an intracutaneous injection he showed no reaction and 10,000 units of antitoxin were injected intravenously. Almost immediately he developed a short hacking cough with difficulty in breathing. The skin seemed puffy, very red and somewhat purplish. These symptoms lasted about five minutes and then subsided abruptly. The patient sank back white and weak. Forty minutes later there was a chill that lasted about ten minutes, the temperature rose to 102°.

During the past two weeks intravenous injections of antitoxin have been given to twelve small children, who had just before that treatment developed after an intracutaneous test small wheals with an areola of about ½ to 1¾ inch in diameter. One of these developed a moderate chill and three a slight chill. Intravenous injections were also given to six developing no reaction. One of these developed a slight chill.

These results allow us to draw certain tentative conclusions. Persons who do not possess skin hypersensitiveness may occasionally develop serious symptoms after an intravenous and even after an intramuscular injection of horse serum but they are less liable to do so than those who have the skin reaction.

Cases which show a moderate reaction after an intracutaneous injection as shown by an areola of one half to two inches may or may not show an accelerated reaction to a therapeutic dose of serum. The reaction is not as a rule marked and almost never dangerous.

Cases which show a marked skin hypersensitiveness are apt to develop within a few minutes a marked serum reaction upon the injection of a therapeutic dose of serum. The reaction from a subcutaneous or an intramuscular injection is not, however, at all likely to be a dangerous one and can be readily controlled. The reaction from an intravenous injection is also not dangerous in the great majority of cases, if the antitoxic globulin solution is given very slowly and is stopped at the first appearance of symptoms. I believe we are justified without waiting for the result of an intracutaneous test in giving an immediate intravenous injection in every severe case of diphtheria unless we see evidences of status lymphaticus or obtain a history of asthma.

Although Hooker's findings are corroborated to a degree by the observations recorded in this paper it should be emphasized again that the sensitization is not as marked as that developing after larger dose of antitoxin for passive immunization. Even in the latter cases as indicated by the personal experiences noted above we need have no especial fear of reinjecting antitoxin or serum. It would seem, therefore, that the lesser sensitization given by toxin-antitoxin was in all probability a nearly negligible factor.

As this report is preliminary in character there seems no need to refer to such analyses of the results of second injections of antitoxin as have been published. The acceleration of the reaction after second injections has been observed by many and this has been studied especially by Von Pirquet and Schick. In one or two instances a fatal result has been attributed to the fact that a previous injection had been given. Notwithstanding this, there remains the fact that practically all of the few deaths following the injection of antitoxin have followed the first injection.

The suggestion of Hooker that a goat antitoxin be available has been made before for passive immunization but not as a substitute for horse serum in toxin-antitoxin. The fact that such an antitoxin is not available is evidence in itself that the experiences with second injections has never been sufficient to raise the fears of physicians and therefore, a demand for such an antitoxin. Its preparation is perfectly feasible in the small amounts that would be required and I have already started its preparation. Not only will there be some advantage in preventing the moderate amount of sensitization which results from the toxin-antitoxin injections, but the removal from the minds of physicians of any fear of this change will be of importance. Further observations on the apparent sensitization are in progress and these experiences will be presented at a later date.

ANTITOXIN IN DIPHTHERIA—A COMPARATIVE STUDY OF THE USUAL METHODS OF ADMINISTRATION WITH THE INTRAPERITONEAL METHOD*

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Ever since the introduction of antitoxin as a specific agent in the treatment of diphtheria, there has been considerable discussion as to the most suitable means of administering it. The methods now in vogue, namely, the subcutaneous, the intramuscular, and the intravenous are used alone or in combination, depending on the severity of the case. Another route not previously employed for this purpose but commonly used in the giving of fluids to infants, and more recently even of whole blood, is the intraperitoneal route.

It is the purpose of this article to set forth the advantages and disadvantages of the respective methods. To do this it will be necessary to consider the following factors: (1) the relative technical difficulties involved; (2) the reaction, local and general; and (3) the rapidity of absorption.

Technique of Administration. It is conceded that the technique of subcutaneous and intramuscular injections is simple. The details are too well known to need repetition. One point should be emphasized, however. In the intramuscular injection it is important to insert the needle deeply into the muscles, otherwise a preparation thought to be in the muscles may have reached only the subcutaneous tissues.

Intravenous administration of antitoxin unfortunately is more difficult, and particularly so at the ages when diphtheria is most common. No doubt the skill of the operator modifies this factor to a certain degree but even in expert hands it may be a real problem. It was this inability to find suitable veins in infants with laryngeal and toxic diphtheria, where rapid absorption is so essential, that first prompted a search for another method—one which would afford simplicity of technique as well as rapidity of absorption.

The absorptive power of the peritoneum previously shown by many observers suggested this area as a possible route for antitoxin and since the safety of the procedure had been attested to by many, it remained only to be demonstrated that the serum itself was non-irritating and that its absorpion would warrant the use of the method in selected cases.

The technique of intraperitoneal injection is, of course, simple and has been well described by Blackfan: "The patient lies in the recumbent posture with the movements of arms and legs restricted by a restraining jacket. The most favorable site for the introduction of the needle is through the linea alba just below the umbilicus. The skin is prepared after the usual surgical method, with iodin and alcohol, and the area may be rendered anesthetic with ethyl chlorid. The solution is introduced by gravity from an infusion bottle. When the patient is restrained in the proper position and the site for the injection prepared, the skin and subcutaneous tissues are picked up between the thumb and the index finger and the needle, pointing upward, is inserted at an oblique angle. After it has penetrated the peritoneum the fluid is allowed to flow into the peritoneal cavity."

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*From the Willard Parker Hospital and the Research Laboratory.

Reaction. The local and constitutional symptoms following any method will of course be influenced by the type of the preparation of antitoxin used. This is well illustrated by the figures of Park, showing the advantage of refined antitoxin (antitoxic globulin solution) in 50 treated cases of diphtheria.

TABLE 1.
(PARK)

	Children treated with the whole serum. Per cent.	Children who were treated with the antitoxin globulins. Per cent.
Marked constitutional symptoms accompanied by a severe and persistent rash	28	0
Moderate constitutional symptoms accompanied by a well-developed erythema or urticaria	18	4
Very slight constitutional disturbance accompanied by a more or less general urticaria or erythema	20	8
No appreciable constitutional disturbance but more or less general urticaria or erythema	4	34
No appreciable deleterious after-effects whatever	30	54

	Duration of Rashes								Totals
Days	1	2	3	4	5	6	7	8	
Antitoxic globulin cases	5	7	5	2	3	—	1	—	23
Whole serum cases	1	4	10	1	10	3	2	5	36

In making this study, therefore, the qualitative factor was minimized by the use in all cases of the refined product of the Department of Health Laboratories, City of New York.

After subcutaneous and intramuscular injections there is usually more or less localized tenderness which lasts about two days. A small number of patients may show mild latent reactions associated with urticaria but ordinarily there are no appreciable constitutional symptoms.

The intravenous method causes, of course, no local discomfort, providing there is no escape of the serum into the surrounding tissue. The important consideration in giving it intravenously then is the degree of constitutional reaction. This was formerly a serious hazard but has been diminished to such a degree that today the method is used frequently on our diphtheria service with only occasional severe reactions—a fact directly attributable to the improvement in the product of the laboratory. In spite of careful precautions, however, there is a certain number of cases in which chills, hyperpyrexia and even convulsions occur. In the child already very sick with diphtheria, the question arises as to the wisdom of adding the possibility of such deleterious effects. Of course in a large number of cases the advantage of the immediate presence of antitoxin in the blood unquestionably overbalances not only this danger but also the harm which may ensue from waiting for absorption from the muscles or skin.

The intraperitoneal method, first tried out on rabbits, has, up to date, been given in twelve clinical cases. The patients varied in age from five months to fourteen years (the older ones being used to facilitate the collection of blood specimens). In none of these patients was there any evidence of local discomfort. One child developed slight urticaria on the seventh day but none showed any constitutional symptoms. The greatest temperature rise in any case was .8° F. In four there was no change, whereas in two an actual drop occurred. The average rise for twelve cases was .3° F.

Although the number of patients is too limited to make any accurate statement concerning general reactions, the indication is that these approximate the subcutaneous and intramuscular rather than the intravenous.

Absorption. It is this factor which after all is of first importance, not only in advanced cases, but also in those defined as mild because of the limited involvement. A small membrane not infrequently proves a false prognostic criterion in predicting subsequent paralyses.

Method of Estimation. To compare any one method with another from the standpoint of absorption, it is essential that we have an accurate measure. This is furnished by the quantitative antitoxin determination, according to Hygienic Laboratory Standards, on blood taken before and at stated intervals after injection.

Serum from the blood of an animal or man to be tested is mixed with a toxin of known potency and injected into a guinea pig. The quantity of serum is varied according to the amount of antitoxin to be tested for and retests are performed until it is demonstrated that a definite quantity of the serum protects against the test dose of toxin. From this is computed the amount of antitoxin per c.c. of serum.

Natural Antitoxin. An individual to be immune to diphtheria must have at least 0.03 units of antitoxin per cc. present in the blood. Theoretically of course a person contracting diphtheria is Schick positive and possesses no antitoxin. It has been demonstrated, however, that a considerable percentage of persons have developed some antitoxin by the end of the second or third day following invasion of the body by the infecting organisms.

With the view of determining the extent of the presence of antitoxin as well as avoiding any error in regard to the receipt of it before admission to the hospital, tests were carried out on fifteen initial bleedings. The results are given in Table 2.

TABLE 2.

AMOUNT OF ANTITOXIN PER CC. OF BLOOD SERUM PRESENT IN FIFTEEN CASES WITH DIPHTHERIA OF VARIOUS TYPES BEFORE INJECTION

Case No. 5.	None.
Case No. 6.	None (toxic).
Case No. 7.	0.04 units.
Case No. 8.	0.04 units.
Case No. 9.	0.07 units.
Case No. 10.	None (toxic).
Case No. 11.	0.06 units.
Case No. 13.	0.09 units.
Case No. 14.	0.04 units.
Case No. 15.	0.07 units.
Case No. 16.	None.
Case No. 17.	0.12 units.
Case No. 18.	0.07 units.
Case No. 19.	0.125 units.
Case No. 21.	None (toxic).

All types are represented among this group of fifteen, such as carriers, nasal diphtheria and the most severe, neglected forms. Although the figures indicate an appreciable activity on the part of some of these individuals to make antitoxin, the quantities found do not bear out the statements of relatively large amounts occasionally reported. It is possible that some of the latter may be explained by unreliable histories concerning the injection of antitoxin previously.

Rapidity of absorption. *Intravenous.* The time element in this method is of course eliminated.

Table 3 shows the amount of antitoxin per cc. of blood serum present at different intervals after intravenous administration of 10,000 units.

TABLE 3.

(AFTER PARK & WILLIAMS)

5 minutes after	13.6 units
24 hours after	11.0 units
3 days after	8.9 units
5 days after	7.4 units
7 days after	6.4 units

(See chart also.)

(For purposes of comparison these figures have been corrected according to weight and represent an average of four cases. Throughout this study a standard weight of thirty-five pounds will be maintained.)

Subcutaneous. The figures obtained after subcutaneous injection of 10,000 units are:

TABLE 4.

(AFTER PARK & WILLIAMS)

24 hours after	2.0 units
3 days after	4.4 units
4 days after	3.8 units
5 days after	4.1 units
7 days after	3.4 units

(These figures have also been corrected for weight.)

Intramuscular and Intraperitoneal. The preliminary tests on rabbits showed a rather striking difference between these two methods.

TABLE 5

(COMPARATIVE ABSORPTION IN RABBITS)

Intramuscular	(10,000 units)	Intraperitoneal
2 units per cc. blood serum	2 hours	19 units per cc.
3 units per cc. blood serum	4 hours	30 units per cc.
4½ units per cc. blood serum	6 hours	36 units per cc.

A clinical application was then made on twenty-one cases of diphtheria, twelve of which received antitoxin intraperitoneally and nine intramuscularly.

The twelve cases receiving intraperitoneal injections may be briefly outlined as follows:

Case 1. R. C. two years. Weight, thirty-five pounds, four days ill, membrane covering both tonsils. Bilateral anterior cervical adenitis. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin 4 hours—0.75 units
Recovered. 6 hours—2.0 units

Case 2. I. G. fourteen years. Weight, seventy pounds, four days ill, membrane covering tonsils. Slight bilateral anterior cervical adenitis. 14,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin 1 hour —0.25 units
3 hours—0.33 units
12 hours—1.75 units
Recovered. 24 hours—5.00 units

Case 3. S. J. fifteen months. Weight, twenty-six pounds, five days ill, pharynx negative to inspection. Temperature 104°. Pronounced retractions—complete impairment of phonation—color poor—physical signs of bronchopneumonia—almost moribund. Laryngoscopic examination—marked supraglottic edema—membrane on ventricular bands. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin 3 hours—0.5 units

Intubated one hour after admission. Died thirty-five hours later.

Autopsy (portion of report)—Small needle mark about one inch below umbilicus—no evidence of this on peritoneal surface—no congestion of surrounding tissues. Peritoneum smooth and glistening, free from adhesions. No injection of vessels. Peritoneal cavity contains no free fluid and intestines appear normal. Lungs—consolidation of both lower lobes, left upper and right middle lobes, with many firm nodules—purulent exudate from bronchioles.

Diagnosis—Laryngeal diphtheria and extensive bronchopneumonia.

Case 4. G. M. four years. Weight, thirty-seven pounds. ? days ill—membrane over posterior half of tonsils and posterior pillars. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin 2 hours—0.33 units
4 hours—0.75 units
Urticarial rash on seventh day. 12 hours—2.00 units
No rise in temperature. No constitutional symptoms.
Recovered.

Case 5. S. S. two and a half years. Weight, thirty-eight pounds, five days ill—extensive sloughing membrane over tonsils and uvula—profuse purulent nasal discharge—bilateral anterior cervical adenitis. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin Before—None
2 hours—0.33 units
Recovered. 14 hours—2.50 units

Case 6. H. N. five years. Weight, thirty-nine pounds, five days ill, toxic extensive membrane over both tonsils, pillars, and uvula. Bilateral units with 8-10 cc. saline by gravity.

Blood serum antitoxin Before—None
2 hours—0.5 units
4 hours—0.75 units
Recovered. 18 hours—4.00 units

Case 7. W. W. three and a half years. Weight, thirty-five pounds, three days ill, serous nasal discharge, membrane covering both tonsils, bilateral anterior cervical adenitis. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin Before—0.04 units
5 hours—1.50 units
Recovered. 16 hours—4.00 units

Case 8. R. O. six years. Weight, forty pounds, three days ill, membrane covering both tonsils, bilateral anterior cervical adenitis, soft systolic mitral murmur. 10,000 units with 8-10 c.c. saline by gravity.

Blood serum antitoxin Before—0.04 units
7 hours—1.50 units
Recovered. 16 hours—4.00 units

Case 9. V. C. five months. Weight, fourteen pounds, ? days ill, profuse serosanguineous nasal discharge. 6500 units with 8-10 cc. saline by gravity.

Blood serum antitoxin Before—0.07 units
Recovered. 6 hours—6.5 units

Case 10. W. G. six years. Weight, fifty-five pounds, five days ill, extremely toxic on admission, sloughing putrid exudate over tonsils and pillars, bilateral anterior cervical adenitis, veins small and partially collapsed. 14,000 intraperitoneal with few cc. saline by gravity. Pulse rather weak and thirty-six hours after admission dropped suddenly from 130 to 80. Death twelve hours later.

Blood serum antitoxin Before—None
24 hours—7 units
48 hours—6 units

Autopsy (portion of report)—Peritoneum smooth and free from adhesions—no signs of inflammation.

Summary—Acute hemorrhagic myocarditis, pulmonary edema and congestion. Acute parenchymatous degeneration of liver and kidneys. Congestion of viscera.

Diagnosis—Septic diphtheria.

Case 11. J. D. eight years (?). Weight, forty-six pounds. Positive cultures but no clinical evidence of diphtheria. 10,000 units with 8-10 cc. saline by gravity.

Blood serum antitoxin Before—0.06 units
24 hours—3.00 units
36 hours—6.00 units
Recovered. 72 hours—3.00 units

Case 12. C. H. eight years. Membrane on right tonsil. 10,000 units with 8-10 c.c. saline. No determination.

Of this group of twelve, two were nearly moribund on admission and died with thirty-six and forty-eight hours respectively. Antitoxin was given intraperitoneally to these only because of the inability to give it by vein. They are reported with autopsy findings which show death to be due in one case to laryngeal diphtheria and extensive bronchopneumonia; in the other, to septic diphtheria with hemorrhagic myocarditis. In both, the peritoneum was free from any pathologic condition.

Ten cases recovered without complications and it is interesting to note that in most of these the membrane disappeared at about the same time that it did in a number of intravenous cases observed simultaneously.

The intramuscular group includes cases of nasal diphtheria, very mild tonsillar diphtheria and carriers.

They were selected to conform as nearly as possible in age and weight to the intraperitoneal cases. A brief statement of each is given:

Case 13. G. W. five years. Weight, thirty-six pounds. Positive culture, no clinical evidence of diphtheria, 10,000 units intramuscularly.

Blood serum antitoxin Before—0.09 units
5 hours—0.14 units
7 hours—0.33 units

Recovered.

Case 14. T. O. four years. Weight, thirty-seven pounds. Very small amount of membranous exudate on both tonsils. No adenitis. 10,000 units intramuscularly.

Blood serum antitoxin Before—0.04 units
2 hours—0.10 units
4 hours—0.16 units

Recovered.

Case 15. R. Y. fifteen years. Weight, ninety pounds. Positive culture but no clinical evidence of diphtheria. 10,000 units intramuscularly.

Blood serum antitoxin Before—0.07 units
3 hours—0.07 units
6 hours—0.14 units

Recovered.

Case 16. R. K. three years. Weight, thirty pounds. Positive culture, serosanguineous nasal discharge, no membrane. 10,000 units intramuscularly.

Blood serum antitoxin Before—None
6 hours—0.14 units

Recovered.

Case 17. G. C. eleven years. Weight, fifty pounds. Positive cultures. No clinical evidence of diphtheria. 10,000 units intramuscularly.

Blood serum antitoxin Before—0.12 units
6 hours—0.20 units
12 hours—1.50 units
24 hours—2.50 units
48 hours—5.00 units

Recovered.

Case 18. F. T. five years. Weight, thirty-seven pounds. Serosangineous nasal discharge, positive culture. 10,000 intramuscularly.

Blood serum antitoxin Before—0.07 units
7 hours—0.20 units
24 hours—3.00 units

Recovered.

Case 19. R. G. seven years. Weight, forty-two pounds. No clinical evidence of diphtheria. 10,000 units intramuscularly.

Blood serum antitoxin. Before—0.125 units
12 hours—0.90 units
24 hours—2.00 units
48 hours—3.00 units
Recovered. 72 hours—4.00 units

Case 20. F. K. seven years. Weight, forty-five pounds. No clinical evidence of diphtheria. 10,000 units intramuscularly.

Blood serum antitoxin 24 hours—1.0 units
48 hours—1.5 units
60 hours—2.0 units
Recovered. 72 hours—2.5 units

Case 21. D. McN. No clinical evidence of diphtheria. 10,000 units intramuscularly.

Blood serum antitoxin Before—None
24 hours—1.0 units
36 hours—2.5 units
48 hours—3.5 units
Recovered. 96 hours—3.0 units

On fifty bleedings, taken at various time-intervals from the patients receiving intraperitoneal and intramuscular injections, a total of one hundred and thirty-four tests were performed.

Table 6 gives figures representing in most cases an average of several bleedings.

TABLE 6.

COMPARATIVE ABSORPTION OF ANTITOXIN BY THE INTRAMUSCULAR AND INTRAPERITONEAL METHODS

Intramuscular	Hours	Intraperitoneal
	1	0.38 units per cc. blood serum
0.10	2	0.42 units per cc. blood serum
0.18	3	0.43 units per cc. blood serum
0.17	4	0.79 units per cc. blood serum
0.15	5	1.50 units per cc. blood serum
0.21	6	2.30 units per cc. blood serum
0.34	7	1.70 units per cc. blood serum
1.66	12	2.37 units per cc. blood serum
	14	2.71 units per cc. blood serum
	16	4.00 units per cc. blood serum
	18	4.57 units per cc. blood serum
2.30	24	6.5 units per cc. blood serum
		* * *
2.70	36	7.88 units per cc. blood serum
		* * *
4.20	48	6.75 units per cc. blood serum
		* * *
2,8*	60	
		* * *
4.00	72	3.94 units per cc. blood serum
		* * *
3.2	96	

(The dose was 10,000 units except in two cases. Proper corrections have been made for these as well as for standard weight of thirty-five pounds.)

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*One bleeding only—24 hours—1 unit; 48 hours—1½ units; 60 hours—2 units; 72 hours—2½ units.

Several interesting points can be deduced from the preceding figures. To begin with, the most striking difference in the two methods is manifest in the first seven hours, the time of greatest importance. Within an hour after intraperitoneal injection, the blood is appreciably antitoxic, and up to seven hours the absorption is about five times that of intramuscular.

The peak of absorption for the intraperitoneal method lies apparently in the vicinity of thirty-six hours, whereas for the intramuscular it is somewhere between forty-eight and seventy-two hours. (In cases with successive bleedings it was nearer seventy-two.) From these points the values decrease as the serum is eliminated.

The high figure on the intraperitoneal side at six hours as compared to seven hours is noteworthy. It is explained by Case 9 which was included in the average for six hours. This case was one of nasal diphtheria in an infant of five months, which weighed only fourteen pounds. Although it received only 6,500 units its blood serum at six hours showed an actual antitoxin content of 6.5 units per cc. This figure when corrected for standard weight (thirty-five pounds) to 3.23 is still much higher than those observed in older children and suggests, as might be expected, that the younger the child, the more rapidly will antitoxin be taken up from the peritoneum. This is significant because it is among the younger children that the procedure seems particularly applicable.

To illustrate these points as well as to facilitate comparison of all four methods a graphic chart is appended.

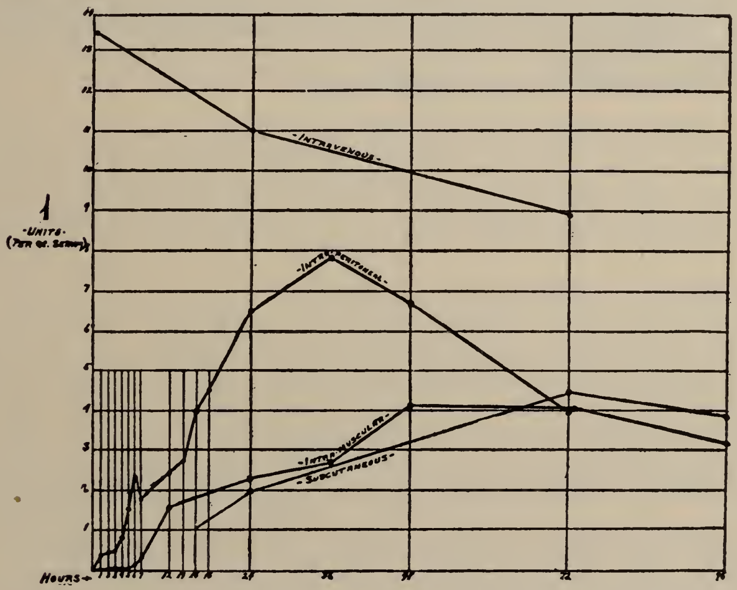

Fig. 1. Graphic representation of absorption—units per cc. blood-serum.

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Summary and Conclusions.

1. A new method of administering antitoxin, namely, the intraperitoneal, is suggested for selected cases of diphtheria.

2. For infants and young children, the simplicity of technique in methods other than the intravenous is emphasized.

3. Local discomfort, an annoying factor in the subcutaneous and intramuscular methods, is absent in the intraperitoneal and intravenous methods.

4. General reactions are noted as a possible hazard, greater in the intravenous than in the other methods.

5. Absorption by the intraperitoneal route does not, of course, approach the intravenous where antitoxin is delivered directly into the blood stream. Early absorption of antitoxin, however, as shown by tests on the blood after intraperitoneal injection, is approximately five times greater than after intramuscular injection. Within an hour it is more than sufficient to neutralize the circulating toxin in the blood of any case of diphtheria.

6. Absorption by the intraperitoneal method is more rapid in infants than it is in older children. It is among the former that the procedure finds its greatest use.

7. Intraperitoneal administration of antitoxin is a safe and simple procedure. It is particularly applicable: (1) In advanced cases where suitable veins for intravenous administration are lacking. (2) In moderately advanced cases in which intravenous injection is not considered necessary or advisable but where more rapid absorption is desired than can be obtained by the intramuscular method.

I wish to express my indebtedness to Dr. Park and Dr. Banzhaf for their kind suggestions and advice in this work and to Mr. Greenwald for his technical assistance.

For bibliography see the original article.

THE EFFECT OF PEPTONE ON THE TOXIGENIC PROPERTY OF B. DIPHTHERIAE NO. 8

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Harriet Leslie Wilcox

It is known to all laboratory workers who are familiar with the production of diphtheria toxin that the strain of B. diphtheriae used in obtaining a potent toxin is called No. 8. This strain was isolated by Dr. Williams in the summer of 1895 and so stable has been its toxigenic property that it is used, not only in the serum laboratories of the United States but in those of England and Europe as well.

The idea that different cultures of this strain should vary in their toxic productions was not entertained up to about 1916. However, from time to time, requests for new transplants of No. 8 had been received at this laboratory from other workers who said that their cultures had lost the ability to produce a potent toxin. Previous to 1914, our strain was still producing a potent toxin, though with some irregularities in strength.

More recently and coincident with the reports from other laboratories, the potency of the toxin produced at the Bureau of Laboratories has shown a marked decline. The average toxicity of the years 1914, 1915 and 1916 was 1:200 (or less) as compared with an average potency of 1:350-1:400 for the years 1911, 1912 and 1913.

In the summer of 1916 I was privileged to work a few weeks at the Pasteur Institute in Paris and to observe the preparation and inoculation of the diphtheria toxin broth. This procedure consisted of seeding Martin peptone broth[1] in Fernbach flasks with fairly large amounts of broth culture of No. 8. This culture had been taken to the Pasteur Institute in 1896 by Dr. Williams. The usual routine for the preparation of diphtheria strain was as follows:

> The stock culture was kept on coagulated ox-serum while the other culture was cultivated entirely on Martin peptone broth and was transferred three times weekly. The culture on serum was subcultured every four weeks and was not used for toxin production unless the broth culture became contaminated; in such case a fresh culture in Martin peptone broth was made from the serum culture. How often recourse to this culture was made, I do not know, but from the careful technic employed one would say, rarely, if ever.

On my return several experimental preparations of diphtheria toxin were made. The method of the Pasteur Institute was used as well as their culture of No. 8 which had been brought back to New York. The results were so superior to those obtained by employing Witte peptone broth and our own culture of No. 8 that in January, 1917, Martin peptone broth inoculated with the Pasteur culture of B. diphtheriae No. 8 was adopted as our method of toxin production. Since we were following as closely as possible the Pasteur method, we used the same procedure for the propagation of the culture of B. diphtheriae.

Comparative tests of our culture of No. 8 with that of the Pasteur No. 8 were made at Dr. Williams' request. The first two or three tests showed such marked difference in favor of Pasteur No. 8 that we decided to discover whether, after a cultivation of several months or longer on Martin peptone broth, our No. 8 culture would produce as high a toxin in Martin peptone broth as the Pasteur No. 8.

To differentiate between these two cultures of the same strain of No. 8 we have called the culture which has been propagated exclusively at the Bureau of Laboratories, Research No. 8, whereas the culture obtained from Pasteur Institute is designated as Pasteur No. 8. These two cultures were inoculated in Martin peptone broth Dec. 28, 1916, and transferred three times weekly in this medium.

At about the same time these cultures were inoculated similarly into Witte peptone broth, Research No. 8 being planted Dec. 23, 1916, and Pasteur No. 8 Dec. 28, 1916, and were transferred three times weekly.

In the summer of 1919 a second culture of No. 8 was obtained from the Pasteur Institute and this was planted Aug. 19, 1919, in Martin peptone broth, Berna peptone broth and Witte peptone broth; it was designated as Pasteur 2 No. 8.

The records of these cultures, that is, the date each was planted in a different medium, the number of transfers weekly, together with the date and result of the planting to determine purity, were kept on separate cards.

In order to obviate any possibility of the substitution of one medium for another while making the subcultures, the following procedure was adopted:

The cultures Research No. 8, Pasteur No. 8, and Pasteur 2 No. 8 in the Martin peptone broth were kept in a different container than the same cultures in the other peptone broths. At the time of transferring only one container with its respective broth tubes was on the work table, thus avoiding any possibility of a Witte peptone broth tube being substituted for a Martin peptone broth tube and vice versa.

The dilutions of the filtered cultures for the animal inoculation were made in 0.8% NaCl. In the tables in which the toxicity is given as being below 1:100 or 1:200, etc., it indicates that those were the dilutions inoculated and that they did not kill the test animals, or if they did so, not within the time limits. When a toxin is stated to be of a given strength it means that the given dilution killed a 250 gm. guinea-pig within the four days with the classical toxic symptoms. Occasionally it was not possible to obtain pigs weighing 250 gm., but in those tests made with heavier pigs, the surplus weight was always discounted in estimating the toxicity. No guinea-pigs weighing below 230 gm. were inoculated.

Martin Peptone Broth

The comparative tests of Research No. 8 and Pasteur No. 8 in Martin peptone broth are given in table 1. It is to be recalled that the cultures were subjected to cultivation in this broth, were transferred three times weekly and were then cultivated under identical conditions. To give the reader the

TABLE 1.

TOXICITIES PRODUCED IN MARTIN PEPTONE BROTH BY THREE DIFFERENT CULTURES OF B. DIPHTHERIAE NO. 8 AFTER CULTIVATION IN THE SAME BROTH; TRANSFERS MADE THREE TIMES A WEEK

Date of Test	Research No. 8 Cultivation Begun Dec. 28, 1916	Pasteur No. 8 Cultivation Begun Dec. 28, 1916	Pasteur 2 No. 8 Cultivation Begun Aug. 19, 1919
Jan. 19, 1917	1:100	1:400	Not tested
May 31, 1917	Below 1:200	1:400	Not tested
June 30, 1917	1:300	1:500	Not tested
Oct. 11, 1917	Below 1:100	1:300	Not tested
Oct. 19, 1917	1:150	1:800	Not tested
Feb. 2, 1918	1:100	1:200	Not tested
Sept. 19, 1919	1:200	1:600	1:600
Oct. 2, 1919	Below 1:100	Below 1:200	Below 1:200

approximate number of generations before the different tests were made, the date of starting the cultures in the different broths is given in the tables.

It will be seen that in one test only Research No. 8 approximated Pasteur No. 8 in its toxic production. In the experiment dated May 31, 1917, Research No. 8 gave a toxicity of 1:300 as compared with a toxin of 1:400 produced by Pasteur No. 8. The greatest difference in their toxigenic powers was shown in the test dated Oct. 19, 1917, when the M L D of Research No. 8 was 1:150 in contrast to a potency of 1:800 produced by Pasteur No. 8. Subsequent comparative tests showed a diminution in the toxigenic production by both cultures in Martin peptone broth.

The first test comparing the three cultures, Research No. 8, Pasteur No. 8 and Pasteur 2 No. 8, was made Sept. 19, 1919. The results showed that the two Pasteur cultures gave a toxicity of 1:600 whereas the Research No. 8 gave a low M L D of 1:200. The last comparative test in Martin peptone broth was made in October, 1919, but the toxicities in this test were so low that only negative results were obtained.

Witte Peptone Broth

The number of tests made using Witte peptone broth was limited, due to the scarcity of the peptone. The broth used in these tests was made according to the formula recommended by Theobald Smith for obtaining a potent toxin. As in the test made with Martin peptone, the three cultures were transferred in the Witte broth 3 times weekly. The period of cultivation may be judged from the dates in table 2 in which the results are given.

TABLE 2.

TOXICITIES PRODUCED IN WITTE PEPTONE BROTH BY THREE DIFFERENT CULTURES OF B. DIPHTHERIAE No. 8 AFTER CULTIVATION IN THE SAME BROTH; TRANSFERS THREE TIMES A WEEK

Date of Test	Research No. 8* Cultivation Begun Dec. 23, 1916	Pasteur No. 8 Cultivation Begun Dec. 28, 1916	Pasteur 2 No. 8 Cultivation Begun Aug. 19, 1919
Jan. 19, 1917	Below 1:100	1:400	Not tested
Oct. 26, 1917	1:150	1:700	Not tested
Sept. 15, 1919	1:100	1:400	1:250
Mar. 12, 1920	Below 1:100	Below 1:100	Not tested
May 27, 1921	Below 1:100	Below 1:200	1:450

* This culture had been carried on in Witte broth since 1895 and transferred every other day as the routine procedure.

It will be noticed that despite the fact that Research No. 8 had been cultivated in a medium containing Witte peptone practically ever since its isolation in 1895, its toxigenic property was much less in the first three tests than that of Pasteur No. 8. The test made May 27, 1921, was a great surprise for it was the first time that Pasteur 2 No. 8 had given a higher potency than Pasteur No. 8. Whether this difference would have been demonstrable in the March 12, 1920, test it is impossible to say, but it is evident that the toxigenic property of Pasteur No. 8 in Witte broth had deteriorated. Further reference to this will be made in the following pages.

BERNA PEPTONE BROTH

It was stated in the foregoing that Martin peptone broth was used during the year 1917 at the Bureau of Laboratories for the routine production of diphtheria toxin, with satisfactory results. In the early months of 1918, however, the potency of the toxin was irregular, and in the late spring several successive preparations of the broth gave too low a toxin for practical use. At this time we had just begun to use the Berna peptone for the production of tetanus toxin, and the idea occurred to us that this peptone might be satisfactory for obtaining a potent diphtheria toxin. Therefore a small amount of broth was made according to the method of Theobald Smith, but with the Berna peptone instead of Witte. The result of this test was very gratifying as a M L D of 1:350 was obtained. No comparative tests of Research No. 8 and Pasteur No. 8 were made at this time as I began a year's leave of absence. During this time all of the cultures in the different mediums were carried out on by Miss Alice G. Mann whose cooperation is greatly appreciated. The two cultures, Pasteur No. 8 and Research No. 8 were planted in Berna peptone broth and were transferred three times weekly.

On my return from France in 1919 the comparative tests in Berna peptone broth of the two cultures, Research No. 8 and Pasteur No. 8 were made, together with the second culture of No. 8 that had been obtained from the Pasteur Institute in the summer of 1919 and which is spoken of in the test as Pasteur 2 No. 8. The results are given in table 3.

TABLE 3.

TOXICITIES PRODUCED IN BERNA PEPTONE BROTH BY THREE DIFFERENT CULTURES OF B. DIPHTHERIAE NO. 8 AFTER CULTIVATION IN THE SAME BROTH; TRANSFERS THREE TIMES A WEEK

Date of Test	Research No. 8 Cultivation Begun May 4, 1918	Pasteur No. 8 Cultivation Begun May 4, 1918	Pasteur 2 No. 8 Cultivation Begun Aug. 19, 1919
Aug. 22, 1919	Below 1:100	1:100	1:100
Sept. 13, 1919	Below 1:100	1:250	1:250
Sept. 15, 1919	1:100	1:500	1:500
Sept. 19, 1919	Below 1:100	1:500	1:400
Sept. 26, 1919	Below 1:100	1:100	1:100
Oct. 4, 1919	Below 1:100	1:100	1:100
Oct. 10, 1919	Below 1:100	1:250	1:250
Oct. 18, 1919	Below 1:100	1:150	Below 1:100

Four of the 8 tests given in table 3 showed decidedly the greater toxigenic property of the two Pasteur cultures over that of Research No. 8. The toxicities of the other four tests were so uniformly low that less definite comparisons were possible.

The toxin made from the Berna peptone broth was so inferior from October, 1919, through to the new year that efforts were made to discover whether there was a domestic peptone on the market which would be more satisfactory for diphtheria toxin production. A comparative test of Parke-Davis peptone and Berna peptone broths was made and inoculated with only Pasteur No. 8. The results were encouraging for the toxin made from

Parke-Davis broth had a M L D of 1:700 in contrast to a M L D of 1:200 obtained with Berna peptone broth. Accordingly, the three cultures were cultivated in broth made with Parke-Davis peptone. However, no comparative tests of the toxigenic powers were made until March, 1920. These and subsequent results are given in table 4 in which is also included three tests of the culture "American."

TABLE 4.

TOXICITIES PRODUCED IN PARKE-DAVIS PEPTONE BROTH BY THREE DIFFERENT CULTURES B. DIPHTHERIAE NO. 8 AFTER CULTIVATION IN THE SAME BROTH; TRANSFERS THREE TIMES A WEEK

Date of Test	Research No. 8 Cultivation Begun Jan. 1, 1920	Pasteur No. 8 Cultivation Begun Jan. 1, 1920	Pasteur 2 No. 8 Cultivation Begun Jan. 1, 1920	"American"*
Mar. 3, 1920.....	1:100	1:1000	Not tested	Not tested
Mar. 12, 1920.....	1:100	1:800	Not tested	Not tested
Nov. 16, 1920.....	1:300	1:900	Not tested	Not tested
Nov. 26, 1920.....	1:200	1:1000	1:700	1:1000†
Jan. 21, 1921.....	1:200	1:1000	1:300	1:700‡
April 7, 1921.....	1:100	1:1500	1:700	1:800

* This culture was obtained from Dr. Durand, Lyons, France, and was the B. diphtheriae No. 8 which had been sent him probably from the Pasteur Institute, Paris.

† This culture had been inoculated into Parke-Davis peptone broth for seven generations before seeding of toxin flasks.

‡ This had been in Parke, Davis broth for five generations before seeding of toxin flasks.

The marked differences in the toxigenic power of these strains were again in evidence. As far as was known, there was no explanation for the low toxicity of Pasteur's 2 No. 8 in the test made Jan. 21, 1921. This was 1:300 as against the M L D of 1:1,000 produced by Pasteur No. 8. In all the other comparative tests with the different peptones, the toxin made from Pasteur 2 No. 8 had corresponded very closely with the toxins made from Pasteur No. 8 with the exception of the experiment of Sept. 15, 1919, made in Witte peptone broth (table 2). Here Pasteur No. 8 produced a M L D of 1:400 as compared with a toxicity of 1:250 made from Pasteur 2 No. 8.

It is interesting to note that the "American" culture (table 4, No. 26, 1920) gave after only seven generations in Parke-Davis peptone broth a toxin with as high a potency as the Pasteur No. 8 which had been cultivated eleven months in that medium.

When it was evident that Research No. 8 produced consistently a very much lower toxin than either of the other strains, regardless of the length of cultivation in any particular broth, the thought occurred to us that it would be interesting to see what effect the different peptones may have had on the toxigenic power of Pasteur No. 8 after its cultivation in the different peptone broths. Accordingly, a preparation of Berna peptone broth was inoculated on July 23, 1920, with the Pasteur No. 8 culture which had been cultivated exclusively in Martin, Witte, Parke-Davis and Berna peptone broths. The day before the test was started, seed cultures in Berna peptone broth were planted from these cultures, as fairly large amounts of the seed cultures were used to inoculate the toxin flasks and we wished to obviate carrying over any of the other peptone broths into the Berna broth. The results of this test, together with the dates of first cultivation in the different broths, are given in table 5.

TABLE 5.

TOXICITIES PRODUCED IN BERNA PEPTONE BROTH BY PASTEUR NO. 8 AFTER CULTIVATION IN BROTHS MADE WITH DIFFERENT PEPTONES

Transplanted Three Times Weekly		Date of Test	Toxicity
First Transplant of Series	Cultivated in Broth with		
May 4, 1918	Berna peptone broth	July 23, 1920	1:500
Dec. 28, 1916	Witte peptone broth	July 23, 1920	Below 1:100
Dec. 28, 1916	Martin peptone broth	July 23, 1920	1:450
Jan. 1, 1920	Parke-Davis peptone broth	July 23, 1920	1:450

TABLE 6.

TOXICITIES PRODUCED IN WITTE PEPTONE BROTH BY PASTEUR NO. 8 AFTER CULTIVATION IN BROTHS MADE WITH DIFFERENT PEPTONES

Transplanted Three Times Weekly		Date of Test	Toxicity
First Transplant of Series	Cultivated in Broth with		
Dec. 28, 1916	Witte peptone broth	Dec. 9, 1920	1:150
Dec. 28, 1916	Martin peptone broth	Dec. 9, 1920	1:300
May 4, 1918	Berna peptone broth	Dec. 9, 1920	1:500

The results of the foregoing test show that the cultures of Pasteur No. 8, which had been cultivated exclusively in Berna, Martin and Parke-Davis peptone broths, gave satisfactory toxins. The culture, however, which had been transferred in Witte broth since Dec. 28, 1916, gave a potency of below 1:100.

Another test was made with these same cultures which had been cultivated in broths made with Witte, Berna and Martin peptone, but instead of the Berna peptone broth, Witte peptone broth was used. As in the former test, the seed cultures were planted in Witte peptone broth before the inoculation in the Witte toxin broth.

Here again the culture which had been in Witte broth gave the lowest toxicity. This test was repeated nine months later, on April 22, 1921 (table 7).

TABLE 7.

TOXICITIES PRODUCED IN WITTE PEPTONE BROTH BY PASTEUR NO. 8 AFTER CULTIVATION IN BROTHS MADE WITH DIFFERENT PEPTONES

Transplanted Three Times Weekly		Date of Test	Toxicity
First Transplant of Series	Cultivated in Broth with		
May 4, 1918	Berna peptone broth	April 22, 1921	1:800
Dec. 28, 1916	Witte peptone broth	April 22, 1921	Below 1:100
Dec. 28, 1916	Martin peptone broth	April 22, 1921	1:700
Jan. 1, 1920	Parke-Davis peptone broth	April 22, 1921	1:700

It will be seen that these results confirm the former test in the Witte peptone broth and also that in the Berna broth, in that the culture of Pasteur No. 8 which had been cultivated in the Witte broth gave the lowest toxicity.

Attempts will be made to confirm the results reported in this paper: (1) By subcultivating in Witte broth the culture Pasture No. 8 which has been propagated continuously in Parke-Davis peptone broth since January, 1920, and which has given regularly a potent toxin in this medium; and by comparing these two cultures from time to time in order to determine whether the toxigenic powers of the culture in Witte broth has been adversely affected while the culture kept in Parke-Davis peptone broth retains its ability to produce strong toxin.

(2) By subcultivating Pasteur No. 8, which has apparently lost its toxigenic power by cultivation in Witte broth, in Parke-Davis peptone broth to determine whether this culture after varying lengths of time in Parke-Davis broth may not regain its power to produce a potent toxin.

(3) By further cultivation of Pasteur 2 No. 8 in Witte broth to determine whether this culture will lose its toxigenic power in Witte peptone broth as Research No. 8 and Pasteur No. 8 have apparently done.

SUMMARY

The culture of research No. 8 which has been propagated exclusively in Witte broth since 1895 at the Bureau of Laboratories has apparently lost its power to produce a potent toxin in any of the broths tested.

The Pasteur cultures of No. 8 which were planted in Witte broth Dec. 28, 1916, has also apparently lost its ability to produce a potent toxin in both Witte and Berna peptone broths. The same culture kept in Berna, Martin and Parke-Davis peptone broth produces strong toxins in both the Berna and Witte broths.

The cultures of Research No. 8 which have been carried on in Berna, Martin and Parke-Davis peptone broths have not as yet recovered their powers to produce a potent toxin in any of the broths tested.

The toxigenic property of Pasteur 2 No. 8 culture has not as yet been affected deleteriously by the two years' cultivation (1919-1921) in Witte peptone broth.

CONCLUSIONS

It would seem in view of the foregoing results:

That Witte peptone broth had an inhibitory or destructive influence on the toxigenic powers of both Research No. 8 and Pasteur No. 8 cultures.

That Pasteur 2 No. 8 may be similarly affected after a still longer cultivation in Witte broth.

That the different preparations of Witte peptone received in this country just prior to 1914 may be responsible for this deleterious effect on the cultures. The fact that culture Research No. 8 from the time of its isolation in 1895 to 1912-1913 had been cultivated in Witte peptone broth without any signs of the lowering of its toxigenic property, as shown by the production of a potent toxin, tends to confirm this conclusion.

That it is possible that the different preparations of any peptone may vary in their effects on the toxigenic property of B. diphtheriae No. 8.

That the continuous cultivation of a culture of B. diphtheriae No. 8 in the same broth as that used for the toxin production is apparently not necessary for obtaining a potent toxin, is shown by the "American" strain of No. 8, which after only seven generations in Parke-Davis broth gave as potent a toxin in that medium as Pasteur No. 8 which had been cultivated for eleven months in Parke-Davis broth.

For bibliography see the original article.

THE EFFECT OF FREEZING AS REGARDS TOXICITY ON DIPHTHERIA TOXIN-ANTITOXIN MIXTURE

Edwin J. Banzhaf and Chas. K. Greenwald

On January 2, 1922, a number of 50 c.c. bottles of toxin-antitoxin mixture No. 38 were sent to a Brooklyn Public School where Dr. Schroeder was immunizing the children. After the day's work was finished the excess toxin-antitoxin mixture (about four 50 cc. bottles) was left in the school room. The following day she took more of the same preparation to the school and, on reclaiming the four left overs, found them to be frozen solid and had them returned to the laboratory on the morning of January 4. When they arrived they were still frozen solid. We kept them at room temperature until they were thawed. After thawing and shaking they were very turbid.

Tests were put on guinea pigs the same day as follows, and with the following results:

Dose injected	*Reactions*	*Results*
1 cc.	No paralysis	Discharged
1 cc.	No paralysis	Discharged
3 cc.	No paralysis	Discharged
3 cc.	No paralysis	Discharged
5 cc.	Slight paralysis	Dead 23 days
5 cc.	Slight paralysis	Dead 27 days Pneumonia

Preparation No. 38 was a five L+ mixture and was ready for Berkefelding April 18, 1921. Tests were put on the following day as follows: The two cc. pigs died from complete paralysis after 24 and 28 days. The five cc. pigs died within 11 and 13 days.

The last test was made on Dec. 15, 1921 on the bottled lot that was used by Dr. Schroeder and was as follows:

Dose injected	*Reactions*	*Results*
2 cc.	Marked paralysis	Recovered
2 cc.	Marked paralysis	Recovered
5 cc.	Complete paralysis	Dead 21 days
5 cc.	Complete paralysis	Dead 21 days

No further tests were made as these convinced us at that time that nothing serious resulted from a solid freezing other than a diminution of the toxicity and also of the immunity value.

On January 8th, 1924, a physician from Atlanta, Georgia, wrote that he had four vials of preparation No. 91 toxin-antitoxin 0.1 L+ that had been frozen and asked for others. We wrote him to return those that were frozen. When the vials were received only a very slight haze could be detected when compared with other vials of the same preparation. Guinea pigs injected with five cc. showed no symptoms of paralysis and on schicking seven weeks later showed no immunity. This preparation No. 91, on Nov. 26, 1923, tested as follows: One cc. pigs, complete paralysis with death in 19 and 21 days. Three cc., death in 9 and 17 days. Five cc., death in four and six days.

On or about the third of February we heard from Massachusetts that a lot of toxin-antitoxin mixture had become toxic after freezing.

We immediately froze a number of our preparations selecting those that were prepared with different lots of toxin. The toxin-antitoxin mixtures which we froze were 3, 1, 0.5 and 0.1 L+.

The procedure in all the freezing experiments in the laboratory were as follows:

One hundred cc. samples were taken and placed 50 cc. in each of two four-ounce bottles which were well stoppered and sealed. As one of each pair was to be frozen they were marked for identification with a diamond pencil. The samples with the remaining amounts were placed in the refrigerator for controls. At about 3 P. M. a 36 quart container was half filled with cracked ice and salt, the bottles placed therein and packed separately with ice and salt and the container then filled with ice and salt. The container cover was adjusted and the whole placed outside on the fire escape. At ten in the morning there was always ice left in the container. The contents of the bottles were always found to be frozen in a solid mass. These were called the 18 hour freezings. Thawing was allowed to take place at room temperature. All the products, after thawing, with the exception of autoclaved media which was ready for planting, showed precipitates. Those of the toxin-antitoxin mixtures were slight for the 0.1 L + and increased with the more concentrated mixtures. On shaking the 0.1 L + showed a very slight haze; the 1 L + a definite turbidity and the 3 L + was very turbid. Tests were made the same day except in cases stated.

The 3 L + mixture that was frozen was prepared in June 1922. At that time one cc. doses caused complete paralysis in guinea pigs and death in from 15 to 23 days. One year later 3 cc. caused paralysis with death in 30 days and 5 cc. doses paralysis with death in 20 to 33 days. After freezing, 5 cc. doses in guinea pigs failed to cause the slightest sign of paralysis. Twenty-five cc. of the frozen mixture was centrifuged, twenty cc. pipetted off and the precipitate rubbed up in the remaining five cc. This was injected into a guinea pig. The pig showed no sign of illness or paralysis. These pigs were schicked seven weeks later and showed no immunity. As this mixture had not been tested for more than seven months, five cc. doses of the sample before freezing were injected. These pigs showed a definite paralysis. A one L + mixture was prepared in November 1922 and was very toxic. One cc. pigs died acutely within three days. After freezing, five cc. injected into guinea pigs failed to show paralysis. Seven weeks later the same frozen sample was retested. Five cc. doses showed no reaction.

Sample before freezing, tested on March 19, 1924, gave the following: One cc. doses, death in 4½ days and 5½ days. Five cc. pigs died acutely in two and a half days. The freezing of this acutely toxic mixture of 1 L + toxin-antitoxin caused a complete loss of toxicity.

A one-half L + mixture was prepared in November, 1922 and was also acutely toxic in guinea pigs with one cc. doses. Tests again December 26, 1923, one cc. pigs died in 10 and 12 days. Three cc. pigs died in 5 days and five cc. pigs in 3 days.

After freezing, 5 cc. doses failed to produce symptoms of paralysis. Schicked after seven weeks the reading showed only a slight immunity. Here again the freezing of a toxic mixture caused practically a complete loss of toxicity.

The tests recorded thus far on freezing were made on mixtures that were more than a year old and in two they were still acutely toxic with 5 cc. doses at the time of freezing.

On February 7, 1924, Dr. Cornman, Director of the Gilliland Laboratory, froze their 0.1 L + toxin-antitoxin mixture No. 40 for 24 hours. After thawing he injected six pigs with one cc. each and six pigs with 5 cc. each. His one cc. pigs showed no evidence of paralysis. All of his five cc. showed some paralysis followed by recovery. At our request he sent the twelve pigs to us. We shicked the 6 one cc. pigs seven weeks after their injection. They showed no immunity. This mixture was prepared in November, 1923, and his last retest was on December 18, 1923, as follows:

Two one cc. pigs showed definite paralysis followed by recovery, two others complete paralysis with death in 26 and 28 days. Three cc. pigs showed complete paralysis and death in 16 and 18 days. Five cc. pigs died in 9 and 12 days.

On February 9 we received from Dr. Benjamin White twenty-four vials of the same 1 L + mixture of toxin-antitoxin which, when frozen in Massachusetts, had become toxic. These vials were labeled No. 101c and each contained 20 c.c. We numbered them from 1 to 24 consecutively. The first two were pooled and likewise the next two. Samples were taken from each pooling and tested. The remainder of each pooling was frozen together with sample of our 1 L + and 0.5 L+ previously reported; a 0.1 L + No. 96, the concentrated toxin used for preparing the mixture and the concentrated antitoxin used in the mixture, all of these were frozen in the same container and in the same manner previously described. The one 1 L + and 0.5 L + again lost their toxicity. The five cc. pigs failed to show any paralysis. Our 0.1 L + No. 96 with two cc. doses showed no evidence of paralysis. One five cc. died in 13 days with lungs consolidated, the other had some questionable symptoms of paralysis and died after 31 days with lungs consolidated. This mixture, No. 96, was prepared November 23, 1923 and tested on the same day as follows: One cc. pigs died in 10 to 14 days; three cc. pigs in four and a half days to five days; five cc. in two and a half days. Tested on February 13, 1924, the sample before freezing killed five cc. pigs in five and a half to eight and a half days. Retested on March 14, one cc. pigs died from complete paralysis in 26 and 27 days. The five cc. pigs died in seven and eleven days. As can be seen the toxicity of this mixture was greatly reduced after freezing.

The concentrated diphtheria antitoxin tested before freezing 1050 units per cc. This is the antitoxin that is being used in preparing our mixtures. After freezing the value remained the same. The aged concentrated toxin sample before freezing had an M.L.D. value of 0.012 and after freezing was 0.07, showing a loss of about 80 per cent. toxin. That diphtheria toxin lost considerable of its value and that diphtheria antitoxic serum retained its value on freezing was known from the work of one of us seventeen years ago.

The Massachusetts 1 L + mixture marked No. 101c gave the following tests before freezing.

	Weight of Pigs in Grams	*Dose in cc.*	*Reaction*	*Results*	*Remarks*
Pooled No. 1 & 2 sample before freezing	245	1	Complete paralysis	Died 26 days	
	255	1	Complete paralysis	Died 19 days	Lungs partly consolidated
	320	5		Died 4 days	Lungs consolidated
	290	5		Died 9 days	
Pooled No. 3 &4 sample before freezing	240	1	Complete paralysis	Died 24 days	
	245	1	Complete paralysis	Died 27 days	
	300	5		Died 11 days	
	310	5		Died 15 days	

These tests proved that before freezing the mixture was just right for the best results for active immunity.

After the freezing the tests were as follows:

	Weight of Pigs in Grams	*Dose in cc.*	*Reaction*	*Results*	*Remarks*
Pooled No. 1 & 2 after freezing	245	1		Dead 6½ days	
	235	1		Dead 6¾ days	
	245	1		Dead 8½ days	
	320	5		Dead 2¾ days	
	280	5		Dead 2 days	
	320	5		Dead 2½ days	
Pooled No. 2 & 3 after freezing	235	1		Dead 6½ days	
	240	1		Dead 1½ days	Lungs consolidated
	250	1		Dead 3 days	Partly consolidated lungs
	310	5		Dead 2½ days	
	320	5		Dead 2 days	
	320	5		Dead 1½ days	

These tests corroborate the findings of Dr. White. He reported that the Massachusetts 1 L + mixture became toxic after freezing.

In order to learn if extreme cold, that is, temperatures below zero Fahrenheit would cause our mixtures to become toxic and what effect it would have on other products, we arranged through the coöperation and courtesy of the Brooklyn Bridge Freezing and Cold Storage Company to have space for prolonged freezing at 16° below zero Fahrenheit.

For this low freezing, we prepared on February 8 a 1 L + mixture with 0.9 unit of antitoxin per L +. Only the five cc. doses were injected into pigs and these died acutely in three and a half and four and a half days. Desiring to have a freshly prepared 1 L + mixture more toxic than this, another was prepared on February 11 with 0.8 of a unit per L +. The one cc. dose pigs died in seven days and the five cc. pigs in two and three days. Samples of these two preparations together with the following were placed in four crates and sent to the cold storage on February 13: samples of an aged diphtheria toxin; a diphtheria toxoid (a toxin with 0.1 % foryrnaldehyde and incubated); a 0.1 L + mixture No. 92 prepared in November; the Massachusetts 1 L + mixture No. 101c, and samples of a 1 L + mixture No. 91 which Miss Kirkbride of the New York State Laboratory sent us.

One crate was removed after a 24 hour freezing, the recorded temperature was 16° below zero Fahrenheit; the second was removed after 48 hours; the third after five days and the fourth after eleven days. As our supply of guinea pigs was very low we could not test the first three crates as they were removed. The samples in the fourth crate were tested the second day after removal. The aged toxin sample lost more than 80 per cent. of its toxin. During December the toxoid (modified toxin) caused with one cc. doses complete paralysis and death in 25 days. After freezing one cc. doses caused symptoms of paralysis with recovery.

The 0.1 L + mixture No. 92 tested on December 10 as follows: one cc. pigs died in 15 to 18 days, three cc. pigs five to seven days, and the five cc. pigs in two and a half to five and a half days. After the eleven days freezing, five cc. pigs failed to show convincing symptoms of paralysis.

The freshly prepared 1 L + mixture with 0.9 unit per L +, after freezing, lost considerable toxicity. The five c.c. dose pigs died in 22 to 26 days. Both pigs showed lungs partly consolidated. Both showed, however, definite symptoms of paralysis. Samples of this mixture before freezing were tested again on March 26, 1924. The five cc. pigs died in 5 to 14 days The second freshly prepared 1 L + mixture with 0.8 unit per L + also lost considerable toxicity. The five cc. pigs developed complete paralysis and died after 25 and 27 days. Samples of this mixture before freezing were retested on March 26, 1924. The five cc. doses killed acutely in three and three and a half days.

The 1 L + New York State Laboratories mixture No. 91 tested on February 11, showed the following results: One one cc. pig died in 14 days, lungs consolidated. Two one cc. pigs died in 39 days. One five cc. pig died in 14 days, the other became completely paralyzed and died in 24 days. After the eleven-day freezing, the five cc. pigs showed some definite paralysis with recovery.

The Massachusetts 1 L + after the eleven-day freezing at 16° below zero Fahrenheit was even more toxic than with the 18-hour laboratory freezing. Only doses of 1 cc. were given, the results follow; at the same time these tests were made a duplicate number of pigs were injected in the leg

No. on Sample 101 c.	*Weight of Pigs in Grams*	*Dose in cc.*	*Results*
9	245	1	Dead 3½ days
9	250	1	Dead 2½ days
10	250	1	Dead 3½ days
10	250	1	Dead 2½ days
11	250	1	Dead 3 days
11	255	1	Dead 2½ days
12	250	1	Dead 2½ days
12	250	1	Dead 3½ days
9	245	1 c.c. + 100 units in leg, no reaction	
9	245	1 c.c. + 100 units in leg, no reaction	
10	240	1 c.c. + 100 units in leg, no reaction	
10	250	1 c.c. + 100 units in leg, no reaction	
11	250	1 c.c. + 100 units in leg, no reaction	
11	255	1 c.c. + 100 units in leg, no reaction	
12	235	1 c.c. + 100 units in leg, no reaction	
12	245	1 c.c. + 100 units in leg, no reaction	

muscle with 100 units of antitoxin and the one cc. test dose injected subcutaneously in the usual place.

Unfortunately immediate further tests could not be made as there was a shortage of guinea pigs. It was our desire to conserve those we had to determine if diluted antitoxin lost any of its value on freezing. We therefore diluted antitoxin with saline solution to three units per cc. to correspond to the number of units in the old 3 L + mixture and also made a dilution with saline solution to one unit per c.c. to correspond with the amount in the 1 L +. We also heated to 80° C for twenty minutes, the toxin used in our mixtures and diluted this with saline solution to conform to a 3 L + per cc. and added three units of antitoxin per cc. A dilution was also made to contain 1 L + and one unit was added per cc. to correspond to the amount of antitoxin in a 1 L + mixture.

After freezing and thawing, a small amount of a light fluffy precipitate was found in the saline dilution containing 3 units per cc. and a smaller amount of a like precipitate in the one unit per cc. Both solutions were shaken and placed in the refrigerator over night. The next day a small amount of the clear solution was removed and the remainder shaken. Both the clear and the turbid solutions of each dilution were tested. The clear solutions and the slightly turbid solutions showed the same antitoxic value as before freezing, proving that the small amount of precipitate was not antitoxin.

Samples of the diluted heated toxin solutions containing three units and one unit of antitoxin per c.c. before freezing, tested in both solutions 15 per cent. under the value of the antitoxin added, proving that even though the toxin had been heated to 80° C. for twenty minutes it still retained some neutralizing value. After freezing and thawing, both solutions had precipitates; these solutions were shaken and placed in the refrigerator to allow the precipitates to settle. The next day, a small amount of the clear solutions was removed and the remainder shaken. Both the clear and the turbid solutions of each dilution were tested.

The three units per cc. heated toxin solution showed a loss of about 30 per cent. of the antitoxin and the one unit solution 25 per cent. loss through freezing. The clear and turbid solutions of both dilutions were of the same value, proving that the precipitate was not antitoxin. Whether the freezing caused a direct actual destruction or caused a further combination of the antitoxin with the heated toxin we can not state at the present time. We are inclined to believe it is a further combination, as the freezing of the antitoxin diluted to the same value with saline solution suffered no loss. This work was repeated with another heated toxin with like results.

A diphtheria toxin was diluted with two parts of saline solution and frozen. Before freezing the strength was 0.033 and after freezing, tests showed it lost 90 per cent. of its strength. Three weeks after the eleven-day freezing at 16° below zero, Fahrenheit, the Massachusetts 1 L + mixture No. 101c, on retests showed that the acquired toxicity after freezing had been lost and that it now tested as it had before freezing. Some of the one cc. pigs showed definite symptoms of paralysis and four of the one cc. pigs died from 17 to 29 days from complete paralysis. The five cc. pigs died in 9 to 18 days.

Two more 0.1 L + mixtures were frozen, Nos. 100 and 104. No. 100 was prepared and tested on February 15, 1924. The one cc. pigs died from complete paralysis in 18 to 21 days; the three cc. pigs died in six and a half to seven and a half days, and the five cc. in three and a half to four and a half days. No. 104 was prepared and tested on March 13, 1924. The one cc. pigs died in 12 to 18 days and the three cc. pigs died in four to five days. The five cc. in three and a half days.

After freezing, the five cc. pigs had lost very little weight after five days. This last freezing was done April 10 and the tests after freezing were made April 11. Sufficient time had elapsed to show that these two most recent preparations had lost considerable toxicity. As it was our desire to have correct p.H. readings made on all the products which we froze in order to ascertain if the reaction of the various toxin-antitoxin mixtures varied to any great extent we broached the subject to Dr. Roland Freeman, Jr. and he in turn enlisted the services of Dr. Frances Krasnow of the Department of Biological Chemistry at Columbia University, and it is she to whom we are greatly indebted for the following readings:

Date of preparation	Product No.	pH before freezing	pH after freezing 18 hrs.	**pH after freezing 24 hours	**pH after freezing 11 days
11-9-23	0.1 L + No. 92	7.3			7.3
11-23-23	0.1 L + No. 96	7.3	7.3		
12-8-23	0.1 L + No. 98	7.3			
2-15-24	0.1 L + No. 100	7.3	7.3		
2-28-24	0.1 L + No. 103	7.3			
3-13-24	0.1 L + No. 104	7.3	7.3		
11-23-22	0.5 L + No. 57	7.3	7.3		
11-23-22	1 L + No. 58	7.3	7.3		
2-11-24	1 L + "Special"	7.5		7.5	7.5
	Albany				
	1 L + No. 91	7.6		7.6	7.6
	Mass.				
	1 L + No. 101 C	7.9		7.9	7.9

*Indicator phenolsulphonephthalein.
**Frozen 16° below zero Fahrenheit.

Whether or not these pH values have any significance we cannot state at the present time. On analysis, however, there seems to be an indication that they do. The Massachusetts 1 L + mixture No. 101c gave a pH reading of 7.9 and the toxicity was increased after freezing.

The Albany mixture has a reading of 7.6 and though this mixture was less toxic after freezing it did not entirely lose its toxicity; we failed to learn its date of preparation. This mixture, although sufficiently toxic for immunizing, was less toxic before freezing than any of the recent mixtures we tested before freezing, and although it lost some of its toxicity after freezing the loss was not as great as those of ours that were more toxic.

It tested on February 11 as follows: Two one cc. pigs did in 39 days. These pigs showed late definite paralysis. One five cc. pig died in 14 days; the other in 24 days from complete paralysis. After freezing, the five cc. doses showed some paralysis with recovery.

Next in order is our 1 L + prepared specially for this freezing work on February 11 with 0.8 of a unit per L +. This has a reading of 7.5 and although after freezing it lost considerable of its toxicity the five cc. pigs died from complete paralysis. Comparing the toxicity of this freshly prepared 1 L + and the 1 L + No. 58 on their most recent tests we have the following.

Prepared	1 L + "Special"	1 cc. doses dead 7 days
February	Tested Feb. 11, '24	5 cc. doses dead 2 and 3 days
11, 1924	Retests March 26th	5 cc. doses dead 3 and 3½ days
Prepared	1 L + No. 58	
November	Tested March 19th, '24	
1922		1 cc. doses dead 4½ and 5½ days
		5 cc. doses dead 2½ and 2½ days

After freezing, the special 1 L + caused with five cc. doses complete paralysis with death in 25 and 27 days. Whereas, 1 L + No. 58 after freezing, failed to cause any evidence of paralysis in five cc. doses.

In conclusion we suggest that for the present our pH value of 7.3 phenol sulphonephthalein indicator be taken for all preparations of toxin-antitoxin mixtures. We also suggest that whenever a new lot of aged toxin is required for preparing mixtures, samples of the first mixture should be frozen and tested.

PREPARATION OF MIXTURE OF DIPHTHERIA TOXIN-ANTITOXIN

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By Edwin J. Banzhaf and Charles K. Greenwald

In preparing the toxin-antitoxin mixture used for active immunization against diphtheria, it is essential to have diphtheria toxin of very high potency. The toxin we employ has a minimal fatal dose† of 0.002 cc. to 0.0007 cc. The advantage of using such highly potent toxins is that they may be diluted with salt solution to the toxin strength actually required, lessening in this way the concentration of other substances (meat extractives, pepton, bacillary substance, etc.), which give rise to local and constitutional reactions.

The most important factor in these reactions is the bacillary substance. The presence of this substance is due to the autolysis of the various bacteria which develop during the preliminary process of fermenting the meat infusion, as well as autolysis of the diphtheria bacillus which is grown on the finished medium for the production of toxin. Now, at Dr. Park's suggestion, the chopped meat is boiled with the required amount of water, thereby producing a sterile infusion. It is then inoculated with B. coli, which produces the necessary fermentation. By this procedure the autolytic products are reduced to only those produced in the growth of B. coli and of B. diphtheriae.

The bacillary substance cannot be eliminated by any method at present available. At best, we can only minimize the reactions in those who are hypersensitive to these substances by using only highly potent toxins which, as stated above, will allow of dilution. Other substances which are present in the broth and which contribute to the reactions disappear to a large extent during the period of storage (aging) of the toxin.

The toxin when freshly prepared, is unstable, and deterioration takes place, the rate of deterioration being relatively rapid at first but gradually becoming slower and slower. After six months or longer the toxin approaches a relative stability and is now used for the preparation of the toxin-antitoxin mixture.

Several lots of such toxin are mixed to give a total of about 60 litres. The L plus dose is then determined. The L plus dose is that amount of toxin which, when added to one unit of antitoxin, will give a mixture which injected into a 250 gram guinea pig will cause death on the fourth day. The toxins used contain from four to 6 L plus doses per cc.

As the convenient immunizing dose was set at one cc. for each injection, it was desirable to have all the preparations of toxin-antitoxin mixtures standardized to contain the same immunizing value; we therefore first set the standard at 3 L plus per cc. but later changed it to 0.1 L plus. Each mixture of toxin is consequently diluted with sterile salt solution, containing the required amount of preservative, to the above mentioned standard.

An aged standardized antitoxin is then added to the toxin, one unit of antitoxin for each L plus. The mixture therefore is theoretically toxic as is evident from the definition of the L plus dose given. The use of this proportion insures against a possible over-neutralization. If the mixture is too toxic, more antitoxin can always be added; whereas at present we would discourage the addition of toxin to adjust an over-neutralized mixture.

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† The smallest amount which will kill a 250 gram guinea pig in four days.

The calculated amount of antitoxin to be used is greatly diluted and added slowly, under aseptic precautions, to the toxin which is constantly agitated to insure a complete mixture. This is then allowed to stand for three hours before the preliminary tests are made.

For the preliminary test two groups of guinea pigs are employed. To each of one group is given one cc.; to each of the second, five cc. The injections are made subcutaneously. If the mixture is properly adjusted at this stage, the pigs receiving five cc. will die acutely, whereas those receiving one cc. will show no immediate effect but will develop paralysis after two weeks.

If the mixture is too toxic, more antitoxin is added till the above condition is reached.

The mixture is now filtered through a Berkefeld filter and stored in the refrigerator for stabilizing. During this period the toxicity of the mixture decreases probably through further combination between the toxin and antitoxin and through some deterioration of the toxin itself.

The mixture is now retested using three groups of guinea pigs, injecting one cc., two cc. and five cc. respectively.

The one cc. group should show some local edema at the site of injection and some loss of weight but should not die. The two cc. group will develop paralysis after about 18 days, dying on the twenty-first or twenty-second day. The five cc. group will develop paralysis after about 15 days and die two or three days later.

If these results are obtained, the mixture is ready for use. If not, further adjustments become necessary. Such a mixture retains practically its full immunizing power for at least one year.

As is evident from the above, there is a tendency to a change in the balance between the toxin and antitoxin during storage. Is there a possibility that dissociation ever takes place, that is, that the mixture may become acutely toxic, and therefore dangerous for human use? All our knowledge concerning the combination of toxin and antitoxin speaks against such a possibility. We have retested our toxin-antitoxin mixtures, at first every month, later every two months, carrying such retests over a period of two years and never noticed any increase in toxicity. In fact, the tendency is the reverse, that is, a loss of toxicity varying in degree with different mixtures. As a rule, the power to produce paralysis persists if the mixture is correctly balanced in the beginning.

OBSERVATIONS ON DIPHTHERIA TOXOID AS AN IMMUNIZING AGENT

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W. H. Park, E. J. Banzhaf, A. Zingher and M. C. Schroeder

Both diphtheria toxoid and diphtheria toxin-antitoxin derive their immunizing potency from altered diphtheria toxin. The freshly prepared toxin has such an instant action on the human tissues that only minute amounts can be used, and immunization with it is therefore a long drawn out process.

Diphtheria toxoid was used for animal immunization in the first experimental work in producing antitoxin and it has continued to be used to some degree ever since.

The utilization of a toxin-antitoxin mixture by von Behring led to the use by us and others of toxin-antitoxin rather than toxoid. During the past three years Glenny and Hopkins in London and Ramon in Paris have been suggesting the substitution of toxoid for toxin-antitoxin. Independently of them we had begun to test the value of toxoid preparations.

Diphtheria toxin can be changed to toxoid by various means, such as storage at moderate temperatures, heating to 50 to 54° C. for a few hours, or exposure to chemicals.

The accepted method at the present time is to add 0.2 to 0.3 per cent. of formalin to the toxin, the amount depending on the protein content and the duration of storage in the incubator.

The advantages claimed for the toxoid are that it is simpler to prepare; is more stable; is somewhat more potent, and it contains no horse serum globulin. In our opinion toxoid is no easier to prepare than toxin-antitoxin. Experience and the following out carefully of every detail of a proper method are necessary in both cases. To the inexperienced a toxoid is easier to prepare because it is a single substance instead of two substances and the difficulty of making a proper mixture is avoided.

The question of stability is more debatable. The longer a toxin is stored, and to a certain extent the greater the percentage of change from toxin to toxoid, the less rapid will be any further change.

The toxin used for toxin-antitoxin is really a mixture of toxin and toxoid. If the toxin-antitoxin mixture is made so that when mixed it has just the right toxicity, it will drop off in potency faster than a toxoid preparation, but if it is made with some excess of toxicity and stored for several months before it becomes suitable for use it will then remain as stable or nearly as stable as the toxoid preparations.

The objection that toxin-antitoxin has a minute amount of horse serum globulin, and as shown by Hooker suffices to cause a moderate serum sensitization, has some weight. It can be obviated by following the suggestion of Hooker of using goat antitoxin. Banzhaf has already prepared such a prepration, and this has been used in a number of children by Schroeder and Zingher. Re-tests have not yet been made. We have never seen any dangerous reactions following the use of serum in those who previously had toxin-antitoxin. We feel certain that there is no objection to giving previously injected children an intramuscular injection of serum. We need more experience before being as sure as to the effect of this slight degree of sensitization on the reaction to an intravenous dose. There is a tendency to attempt to use a toxoid or a toxin-antitoxin preparation that has no toxic effect on guinea pigs. This is very effective in animals, but has a decided drawback. Thus the toxoid sent us by Dr. O'Brien from the Burroughs Wellcome laboratory can be given in five cc. doses to guinea pigs, and has remarkable immunizing potency in this dose. In man, however, one-half a cc. is advisable because the pseudo reaction to the bacillus proteins makes too severe a local reaction. It practically is the same as the old three or five L plus preparations. The comparative reactions from one cc. and from one half cc. of the nontoxic toxoid and one cc. of the 0.1 L plus toxin-antitoxin are shown in Table II. Glenny and Hopkins and Ramon believe that the amount of absorption of antitoxin by a preparation is a pretty exact index of its immunizing power.

This may be true as between different preparations made in the same dilutions from the same materials, and as far as possible in the same manner, but it is far from true if used to test preparations made in different ways and of different degrees of toxicity. The larger the amount of the original toxin in the human dose, the greater will be its antitoxic neutralizing value.

Table I shows the relative absorption of antitoxin by a human dose and the relative potency of different toxoid preparations, as judged by the immunizing results in guinea pigs of a single injection followed by a stimulating Schick test at the end of the third week and a final Schick test at the end of the fourth week. A completely negative Schick test is considered as indicating immunity.

TABLE I.

Preparation	Human Dose	Units of Antitoxin bound by the test dose	Toxicity in 250 gram guinea pigs	Human Doses used as test injections in guinea pigs	After one dose and one Schick test percentage of 15 guinea pigs giving a negative Schick reaction at end of four weeks
B. Wellcome Toxoid 1923	0.5 cc.	3.5	5 c.c. no effect	2.0	70
Same 8 mos. later	0.5 cc.	3.5	5 c.c. no effect	2.0	60
B. Wellcome Toxoid 1924 preparation	0.5 cc.	3.5	5 c.c. no effect	1.0	35
Banzhaf 0.25% formalin					
Toxoid 437	0.5 cc.	2.5	5 c.c. sl. paralysis	2.0	76
Toxoid 350	0.5 cc.	2.5	5 c.c.died 18 days	2.0	70
Banzhaf 0.1% formalin					
Toxoid 437Z	0.1 cc.	0.37	0.2 c.c. sl. paralysis	1.0	15
Toxoid 377Z	0.1 cc.	0.20	0.2 c.c. sl. paralysis	1.0	50
Banzhaf heated 54°C. 5 hrs. Toxoid			5 c.c. no effect	1.0	24
Toxin-antitoxin as comparison					
T.A. 110 0.1L plus			1 c.c. paralysis 21 das.	0.5	85
T.A. 69 0.1L plus			5 c.c. paralysis 27 das.	1.0	65
T.A. 86 0.1L plus			1 c.c. paralysis and recovery	0.5	10
T. A. Solution of precipitate from mixing Toxin and Antitoxin					
Precipitate of toxin 377 Banzhaf			1 c.c. kills 30 days	0.2	80
Precipitate of toxin 517 Banzhaf			1 c.c. slight paralysis	1.0	100

The results of these tests indicate that the best toxoid preparations we have at present are nearly, but not quite, as effective as the best toxin-anitoxin preparations. If only the non-specific proteins could be removed, larger doses of toxoid could be used, and even better results obtained.

RESULTS OF IMMUNIZATION OF MAN

One cc. of original toxoid 377 diluted 1 in 14 to 1 in 20, given to 659 children originally Schick positive, when re-tested by Zingher four to six months afterwards gave 448 negative reactions, or 68 per cent. effective results. Schroeder gave three injections of the same preparation to 187 adults with 87 per cent. apparent success. As control tests were not used by Schroeder with the first injections this should be reduced to about 80 per cent.

With toxoid 437, Zingher got better results—1,525 children giving a positive reaction received three injections of one cc. of a 1 to 20 dilution of the original toxoid. At the re-test two and one-half to three months later, 1,342, or 8 per cent. gave a negative reaction.

Comparing the results we find that toxoid 437, containing a larger amount of antitoxin neutralizing substance, gave the better results in man, but the force of this observation is lessened by the fact that in the test on guinea pigs the reverse result was obtained.

The local and constitutional effects of the diluted slightly toxic toxoid as noted by Zingher and Schroeder were very similar to those following the standard 1|10 L plus preparation of toxin-antitoxin of the same toxicity.

The reaction following the use of non-toxic toxoid was wholly due to the proteins, as there was no active toxin present. In Table II a comparison between the Burroughs and Wellcome toxoid and toxin-antitoxin is given.

TABLE II.

COMPARISON BETWEEN THE REACTIONS DUE TO THE SUBCUTANEOUS INJECTIONS OF THE NONTOXIC TOXOID (BURROUGHS WELLCOME & CO.) AND THE STANDARD 1/10 L PLUS TOXIN-ANTITOXIN IN CHILDREN (SCHROEDER).

29 children (Schick + 10 Schick — 19) receiving 1 cc. of toxoid. Subcutaneous reaction + 16; ± 9; ∓ 3; — 1.

23 children (Schick + 9 Schick — 14) receiving 0.5 cc. of toxoid. Subcutaneous reaction + 5: ± 12: ∓ 3: — 3.

29 children (Schick + 5 Schick — 24) receiving 1 cc. standard 1/10 L plus toxin-antitoxin. Reaction + 6; ± 6; ∓ 4; — 13.

Average diameter of the 1 cc. toxoid reaction 5 cm., ½ cc. toxoid 1.7 cm. 1 cc. T-A 1.7 cm. One cc. of undiluted toxoid gives a larger percentage of pseudo-reactions and more intense reactions than the 1/10 L plus T. A. The ½ c.c. dose of toxoid gives a greater number of marked pseudo-reactions but of no greater intensity than the 1/0 plus T.A.

SUMMARY

At the present time toxoid preparations have been developed which are nearly, if not quite, equal to the best toxin-antitoxin preparations. The toxoid preparations which have no toxicity in guinea pigs cannot be properly used in human beings in doses larger than one-half c.c. because of the marked pseudo-reactions. Toxoid that is slightly toxic can be diluted at least ten times and so reduce the amount of proteins. It is a question whether the slightly toxic diluted toxoid preparations are as stable as the nontoxic ones. Intensive experimental work should be done in both toxoid and toxin-antitoxin preparations so as to be able constantly to have thoroughly potent and stable preparations. After one or two years we should be able to decide which has the greater advantages.

For bibliography see the original article.

THE GRADUAL DECREASE OF THE ACTIVE IMMUNIZING VALUE OF TOXIN-ANTITOXIN MIXTURES STORED DURING ONE YEAR

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Edwin J. Banzhaf

About two years ago when the active immunization from the 3 and 5 L + toxin-antitoxin mixtures were compared the results showed that the 5 L + did not give any greater percentage of active immunity in children than the 3 L +. The 5 L + however did give a higher percentage of local and constitutional reactions in children and adults. These were due to the greater amount of protein substances in the mixture. The 5 L + mixtures were made with the aged diphtheria toxin without any dilution other than that due to the addition of the proper amount of antitoxin, whereas the 3 L + mixtures were prepared from the 5 L + toxin, diluted with water to contain 3 L + and therefore had 40 per cent. less of the reacting substances. Even with this lesser amount of reacting substances, the 3 L + mixtures gave annoying reactions. Every known method had been tried to eliminate these substances, but without success. Knowing that the 5 L + did not give a greater percentage of active immunity than the then standard 3 L +, Dr. Park suggested, after testing several different preparations, a 0.1 L + mixture. That is a mixture that would contain only 3⅓ per cent. of the reacting protein substances of the standard 3 L + and would be neutralized with antitoxin so that the mixture would react in guinea pigs exactly like the 3 L + mixtures. The first experimental 0.1 L + mixture was prepared in the early spring of 1922 by diluting the aged toxin with tap water containing 0.8 per cent. sodium chloride and 0.4 per cent. trikresol. Three hours later 0.5 unit of antitoxin was added to each L + and thoroughly mixed, Berkefelded and tested. Two out of three guinea pigs which received one cc. doses died of late paralysis. The third recovered. One five cc. pig died within five days. The reactions in humans with this mixture were in the majority practically nil. The immunity response was equal to that given by the 3 and 5 L + mixtures, and the response in the children was equal to that in the guinea pigs.

Several months later, on retesting the experimental mixture, it was found to be less stable than the standard 3 L + and that it had lost a considerable amount of its immunizing value. Five months after it was prepared guinea pigs showed the following: three cubic centimeters slight symptoms of paralysis; five cubic centimeters paralysis with death in thirty-seven days. Three months later, five cc. gave slight symptoms of paralysis and ten cc. gave definite symptoms with recovery.

This was its only drawback. In order to overcome it considerable work was done to evolve a method of preparing the mixtures in such manner that they would retain their immunizing value at least nine months.

The immunizing values of two preparations made from the same lot of aged toxin which were mixed by different methods were followed for thirteen months.

Preparation 67 was purposely made very toxic and was prepared February 30, 1922, as follows: The required amount of 0.8 per cent. salt diluent containing 0.4 per cent. alcresta was measured into a suitable container. The required amount of toxin was added to the diluent and thoroughly mixed. The desired amount of antitoxin, in this preparation 0.75 unit per L +, was added immediately to the diluted toxin, thoroughly mixed, Berkefelded and tested. The one cc. pigs died within six to nine days and the five cc. within two to two and one-half days. This proved more toxic than intended. To hasten the decrease of toxicity an 8-liter stock bottle of the preparation was kept at room temperature for two weeks and labeled 67a. Tests were put on November 27, 1922. The one cc. pigs developed paralysis and died within twenty to twenty-six days. The five cc. pigs died within two and one-half days.

The second preparation 69 was intended for immediate use and was prepared on November 15, 1922 as follows: The same amount of the same toxin as used for preparation 67 was measured into a liter bottle and the desired amount of antitoxin, in this case 0.8 of a unit per L+, was measured into the liter bottle containing the toxin, the bottle stoppered and thoroughly shaken. The same amount of salt diluent containing 0.5 per cent. phenol as was used in preparation 67 was measured into a suitable container. The concentrated toxin-antitoxin was then added to the diluent with constant stirring. The bottle contents and the diluent were thoroughly mixed and Berkefelded on the same day. Tested on November 22, 1922, the one cc. pigs died from paralysis within twenty-two days. The three cc. pigs within ten and nineteen days and the five cc. within three and one-half to four and one-half days.

Immunization, in guinea pigs were started with these two preparations on December 5, 1922. The average weight of the pigs was 275 grams.

The injections were given subcutaneously as follows:

PREPARATION NUMBER	NUMBER OF GUINEA-PIGS	AMOUNT INJECTED	WEEKLY INJECTIONS
		cc.	
67a	12	0.1	3
67a	12	0.2	3
69	12	0.1	3
69	12	0.2	3

A like series was injected every six to ten weeks with the exception of the last where the interval was thirteen weeks. Eleven to twelve weeks after the first injection, these pigs were Schicked for immunity value.

For readings of partial immunity in those pigs which might not show a negative reading when given the standard dose of 1/50 of an M.L.D. in 0.1 cc. weaker doses were also given.

Each pig received 1/150, 1/100 and 1/50 of an M.L.D. intracutaneously. The final readings were taken four days later. The readings were recorded as follows:

1/150	1/100	1/50	
+ −	+	+	Normal guinea-pig will die with 1 M.L.D.
+ − −	+ −	+	
+ − − −	+ − −	+ −	Slight immunity will survive 1 M.L.D.
−	+ − − −	+ − −	Partial immunity will survive 1½ M.L.D.
−	−	−	Immunized will survive 2 or more M.L.D.

The figures in the following table are given in percentages:

DATE TESTED	PREPARATION 67a				PREPARATION 69			
	Amount	Paralysis	Number of days for death	Remarks	Amount	Paralysis	Number of days for death	Remarks
	cc.				*cc.*			
November 27, 1922......	1	+	20		1	+	22	
	1	+	26		1	+	22	
	5		2½		3		10	
	5		2½		3	+	19	
					5		3½	
					5		4½	
December 27, 1922......	1	+	28		1	+	28	
	1		13		1	+		Recovered
	1	+	18		3	+	16	
	1	+	21		3	+	15	
	3		7		5		12	
	3		6		5		5	
	5		3					
	5		3					
April 4, 1923...........	1	(?)		Recovered	1	(?)		Recovered
	1	(?)		Recovered	1	+	29	
	3	+	25		3	+	21	
	3	+	29		3	+	29	
	5		12		5	+	18	
	5	+	18		5	+	16	
July 13, 1923...........	1	(?)		Recovered	1	+		Recovered
	1	(?)		Recovered	1	+		Recovered
	3	+		Recovered	3	+	26	Recovered
	3	+		Recovered	3	+	26	
	5	+	25		5	+	15	
	5	+	24		5	+	18	
November 23, 1923......	3	(?)		Recovered	3	+		Recovered
	3	(?)		Recovered	3	+		Recovered
	5	+		Recovered	5	+	27	
	5	+	34		5	+	27	

During the time the preparation showed on retesting sufficient toxicity to kill the five cc. pigs acutely, it was considered advisable to Schick earlier than after twelve weeks, in order to catch some of the pigs before evidences of immunity could be detected. The first series was Schicked after eleven and one-half weeks and not one of the series gave the normal test.

The second series were Schicked after eleven weeks and in this all stages were caught from still normal up to complete immunity. As both preparations were showing decreased toxicity the third series was Schicked after eleven and one-half weeks. There is considerably more immunity developed after eleven and one-half and twelve weeks than after eleven and this explains the higher percentage of immunity of series three and four over the second series which was Schicked after eleven weeks. From the fourth series on, the elapsed time for the Schicking was constant and shows the gradual decrease in the percentage of those immunized. In the last series, preparation 67a shows a sharp drop in the per cent. of those completely immunized. This was expected as can be seen in a table in the original article on the retesting of the preparations. The retest on November 23, 1923, showed only questionable evidences of paralysis in those pigs which received three cc. and paralysis with recovery in one of the five cc. pigs.

Preparation 69 retested on the same date still caused definite paralysis in three cc. and death with paralysis with five cc. doses.

From the work reported and other unfinished work it can be said that toxin-antitoxin mixtures which cause paralysis in guinea pigs with three cc. doses and paralysis with death in five cc. doses is still toxic enough for immunizing, and those mixtures which fail to cause death through paralysis with five cc. doses are too weak. Although 67a was more toxic than 69 when the immunization started, it did not retain the toxicity as long as 69. It cannot be stated at this time that the method of mixing preparation 69 is superior to that of mixing 67 in as far as retaining its toxicity is concerned. It is superior, though, in that it takes less time, less handling, and its accurateness can be more easily controlled. Since preparation 69 all our mixtures have been prepared in like manner and are in like manner holding much of their value for at least a year.

PREPARATION NUMBER	THREE DOSES	SCHICK TEST READING (1/150, 1/100, 1/50)			DATE OF FIRST INJECTION DECEMBER 5, 1922; SCHICKED AFTER ELEVEN AND ONE-HALF WEEKS	DATE OF FIRST INJECTION JANUARY 20, 1923; SCHICKED AFTER ELEVEN WEEKS	DATE OF FIRST INJECTION MARCH 3, 1923; SCHICKED AFTER ELEVEN AND ONE-HALF WEEKS	DATE OF FIRST INJECTION MAY 27, 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION JULY 6 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION SEPTEMBER 6, 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION DECEMBER 14, 1923; SCHICKED AFTER TWELVE WEEKS
67a	*cc.* 0.1	−	−	−	78	25	50	30	27	27	10
	0.2	−	−	−	86	58	58	70	66	63	9
	0.1	−	± − −	± −	11	25	16	10	18	9	20
	0.2	− −	± − −	±	14	8	8	10	9	1	27
	0.1	± − −	± −	+	11	42	8	30	28	18	30
	0.2	± − −	± −	±	0	16	25	0	8	18	18
	0.1	± − ±	± +	+ +	0	8	34	30	27	45	40
	0.2	± − ±	± +	+	0	16	8	20	16	18	45

PREPARATION NUMBER	THREE DOSES	SCHICK TEST READING (1/150, 1/100, 1/50)			DATE OF FIRST INJECTION DECEMBER 5, 1922; SCHICKED AFTER ELEVEN AND ONE-HALF WEEKS	DATE OF FIRST INJECTION JANUARY 20, 1923; SCHICKED AFTER ELEVEN WEEKS	DATE OF FIRST INJECTION MARCH 3, 1923; SCHICKED AFTER ELEVEN AND ONE-HALF WEEKS	DATE OF FIRST INJECTION MAY 27, 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION JULY 6, 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION SEPTEMBER 6, 1923; SCHICKED AFTER TWELVE WEEKS	DATE OF FIRST INJECTION DECEMBER 14, 1923; SCHICKED AFTER TWELVE WEEKS
69	cc. 0.1	—	—	—	75	18	75	34	30	25	18
	0.2	—	—	—	88	42	70	82	62	54	42
	0.1	—	+ — — —	+ — —	13	27	0	16	20	25	27
	0.2	—	+ — — —	+ — —	0	8	10	18	12	18	25
	0.1	+ — — —	+ — —	+ —	12	18	16	0	30	34	36
	0.2	+ — — —	+ — —	+ —	12	42	20	0	25	18	16
	0.1	+ — — + —	+ — +	+ +	0	36	8	50	20	16	18
	0.2	+ — — + —	+ — +	+ +	0	8	0	0	0	10	16

THE PREPARATION OF 0.1 L + TOXIN ANTITOXIN MIXTURE

Edwin J. Banzhaf

The media for Diphtheria toxin to be utilized for toxin antitoxin mixtures is prepared in the usual way with the following exceptions:

To every pound of ground fat-free veal, a liter of water is added and placed in a refrigerator over night. The following morning the liquid is collected and boiled. The media is boiled at this stage to kill all organisms except the few heat-resisting spores, thereby practically limiting the bacterial protein soluble substances to the coli and diphtheria bacilli with which it is inoculated later. After boiling, the reaction is corrected to 1.5 per cent. acid to phenolphthalein; 10 cc. of a 24 hour culture of B. coli is added for every liter of media and incubated about 18 hours. One egg for every two liters is used, then boiled and strained. The reaction is corrected to 0.5 per cent. acid to phenolphthalein. One per cent. of peptone is added (Parke, Davis and Company) and 0.5 per cent. sodium chloride. The reaction is again corrected to 0.5, the media filtered into flasks and autoclaved. At the time of planting one per cent. of glucose is placed in each flask.

The diphtheria toxin is prepared in lots of about 16 liters and stored in gallon bottles for at least a year. When wanted for the preparation of toxin-antitoxin mixture a lot is poured into a suitable sterile container and thoroughly mixed and again poured into the gallon bottles, thereby insuring its uniformity. These bottles are properly labelled and numbered and the L+ determined.

Three preliminary toxin-antitoxin mixtures are made, to establish the exact amount of antitoxin to be added to each L+, also to determine whether the toxin lot has, in sufficient amounts, that portion of toxin which causes paralysis. The three preliminary mixtures of eight liters each, containing 0.1 L + are prepared and Berkefelded. These contain the following amounts of antitoxin: the first 0.75 of a unit to each 1 +, the second, 0.77 unit and the third 0.8 unit. The mixture which causes death in from five to ten days with five cc. doses in 300 gram guinea pigs and paralysis with death with one cc. doses in 250 to 260 gram pigs has the proper amount of antitoxin added to each L +. If the five cc. dose pigs die as stated and the one cc. dose pigs show no definite symptoms of paralysis the lot of toxin is considered too low in that portion of toxin which causes paralysis and is unsuitable for toxin-antitoxin mixtures. These three preliminary mixtures are frozen at minus 8° C. for 18 hours. They are then thawed at room temperature and retested for any change in toxicity. We have always found them to be less toxic after freezing. No further freezing tests are necessary for this subsequent preparation made with the same stock lot of toxin.

When the proper amount of antitoxin to be added to the stock toxin is found, subsequent mixtures of multiple amounts of each will give the same desired results providing the same measuring apparatus and the same procedure is followed.

The procedure: The required amount of toxin is accurately measured into a sterile pint bottle. The antitoxin is diluted so that each one cc. contains 20 units. The required amount is accurately measured into a pint bottle containing the toxin. The bottle is stoppered and thoroughly shaken and placed in a refrigerator until the diluent (sterile physiological salt solution containing 0.5% phenol) has been measured into a suitable sterile container. The concentrated toxin-antitoxin mixture is then added with constant stirring and the bottle rinsed with the diluent.

Although our stock lots of toxin have shown no tendency to deteriorate to any extent in their L + value or noticeably to lose that portion of toxin which causes paralysis, we always standardize two stock lots of aged toxin for our mixtures.

In preparing the mixtures we prepare two: one from each lot of stock toxin. The two lots of stock toxin containing about 16 liters each are sufficient for about a year's supply of 0.1 L + toxin antitoxin mixture. After determining the L + value of the lots of stock toxin and establishing the proper amount of antitoxin to be added to the L + no further standardizing is necessary. If in the three preliminary toxin-antitoxin mixtures the results showed that 0.8 of a unit was the proper amount to add to each L + and four to six months later mixtures prepared with this amount prolonged the life of all the guinea pigs receiving five cc. doses beyond the five-day period we would have the mixture used immediately. The next mixture would then be prepared with 0.75 of a unit per L +. Should this mixture prove too toxic it would be stored in a refrigerator until retests proved it ready for use. No attempt is ever made to add antitoxin to an excessively toxic mixture. Another mixture would then be prepared with 0.77 or 0.78 of a unit.

Preparations of mixtures are made in 32 liter lots. The three agate cans of 48 quart capacity each, which are used only for this product, are sterilized by placing into each about 18 liters of a five per cent. phenol solution, the covers adjusted and the whole boiled for 30 minutes. The phenol solution is poured from two of the cans and 35 liters of water containing 0.8% salt added to each can, the covers adjusted and again boiled for one hour. After cooling, 0.5 per cent. phenol is added and the cans placed in a refrigerator over night during the summer months. The following morning the required amounts of the two standardized stock lots of toxin are placed in two sterile one-liter bottles and immediately marked with their proper respective preparation number. The required amount of antitoxin is added to each, and each bottle is then stoppered and shaken. They are put into a refrigerator while the diluents are measured.

The third can containing the phenol solution is emptied and the required amount of the diluent salt solution measured into it and immediately marked with the preparation number, corresponding to its concentrated toxin-antitoxin mixture. The remaining excess of salt solution is discarded and into that can the second amount of diluent is measured and marked with its preparation number. The excess diluent in the third can is now discarded. This can is utilized for the mixing. The concentrated toxin-antitoxin mixture is now added to the diluent corresponding to its number, with constant stirring, and the bottle rinsed with the diluent. As soon as added the mixture is immediately poured into the empty third can and then back again into the original. This is repeated four times. The second bottle of concentrated toxin-antitoxin is now added to its diluent and mixed by pouring in the same manner. Both preparations are immediately filtered through the Berkefeld and tested on the same day. The temperature of the diluent should never be above 22° C.

The time required to prepare, mix and Berkefeld two preparations of 32 liters each, not including the time to sterilize the cans and salt diluent, is less than three hours.

The pH. reaction of the mixtures is adjusted to 7.3 using Phenol Sulphophthalin as the indicator.

THE EFFECT OF PEPTON UPON THE PRODUCTION OF TETANUS TOXIN

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Harriet Leslie Wilcox

Not very long after the outbreak of the European War, laboratory workers realized that a substitute for Witte pepton in bacteriological work would have to be obtained. Many laboratories were sufficiently stocked with this pepton for the first twelve to eighteen months, but in the spring of 1915 it was evident that the supply of Witte pepton in the United States was fast dwindling and that it would soon be unobtainable.

Witte Pepton

At the Bureau of Laboratories we had been using a Witte pepton glucose veal broth with extremely satisfactory results. Those who have had experience in toxin production, particularly tetanus toxin, know that from time to time there will be marked variation in toxicity due to some unknown factor or factors. The same care may be used in the preparation of different lots of broth as well as in the filtration of the toxin, but the results may differ widely. In consideration of this fact it may be worth while to give the different potencies of the toxin made during the last three to four years when using Witte pepton glucose veal broth.

It is the opinion of some workers that the time of year has a definite influence upon the production of potent toxin. The results as given above show that the seasonal factor was of negative importance as far as toxicity was concerned. If we compare the quarterly results for the year 1915 we find that the averages did not vary greatly. The lowest average toxicity of 1:20,000 occurred in the first, third and fourth quarters, while the second quarter gave an average toxicity of 1:27,000.

The differences in the quarterly averages of the year 1916 were so slight that they were scarcely worth mentioning. The third quarter in this period showed the lowest average toxicity of 1:20,000 while the first, second and fourth quarters gave an average toxicity of 1:22,000.

Doubtless if Witte pepton veal broth had been used throughout the year 1917 we should have had a greater variation in the quarterly averages, due to the two extremely potent toxins produced in the third quarter. As far as was known no especial care was taken in the prepartion of these two broths nor in the filtration of these toxins. In lot 127, the usual procedure of testing for 1:15,000 and 1:25,000 was carried out. When the pigs succumbed in less than forty-eight hours, higher tests, namely 1:40,000 and 1:60,000 were made with similar results. Dilutions of 1:80,000 and 1:100,000 were then injected into guinea pigs weighing 350 grams each. The pig receiving the 1:80,000 dilution died between the second and third day, while the pig which received the 1:100,000 dilution succumbed on the fourth day. Since this was the strongest toxin ever obtained at the Bureau of Laboratories, tests were made of the toxin filtrate to preclude any possibility of spores or bacilli having passed thrugh the Berkefeld filter. These tests were negative.

TABLE 1
WITTE PEPTONE

	1914	1915	1916	1917	1918
First quarter	None produced	7 lots 1:25,000 2 lots 1:15,000† 1 lot 1:10,000* 1 lot 1: 5,000 Average toxicity 1:20,000 Excluding*† average toxicity 1:22,000	1 lot 1:35,000 2 lots 1:30,000 2 lots 1:25,000 1 lot 1:20,000 2 lots 1:15,000 1 lot 1:13,000 1 lot 1:10,000§ 1 lot Below 1:5,000** Average toxicity 1:20,000 Excluding§** average toxicity 1:22,000	None produced	1 lot 1:25,000 1 lot 1:45,000 Average toxicity 1:35,000
Second quarter	None produced	5 lots 1:30,000 4 lots 1:25,000 1 lot 1:20,000 Average toxicity 1:27,000	4 lots 1:35,000 1 lot 1:10,000 1 lot 1: 5,000* Average toxicity 1:19,000 Excluding* average toxicity 1:22,000	None produced	None produced

(*Continued on next page*)

(Continued from previous page)

Third quarter	2 lots 1:25,000	1 lot 1:40,000 2 lots 1:30,000 6 lots 1:25,000 3 lots 1:20,000 3 lots 1:15,000 2 lots 1:10,000 1 lot Below 1:5,000‡ Average toxicity 1:20,000	1 lot 1:30,000 3 lots 1:25,000 1 lot 1:20,000 2 lots 1:15,000	1 lot 1:100,000†† 1 lot 1: 60,000 1 lot 1: 25,000 2 lots 1: 20,000	None produced
	Average toxicity 1:25,000	Excluding‡ average toxicity 1:22,000	Average toxicity 1:20,000	Average toxicity 1:45,000	
Fourth quarter	1 lot 1:40,000 5 lots 1:25,000	2 lots 1:35,000 5 lots 1:25,000 1 lot 1:15,000 2 lots 1:10,000 2 lots 1: 5,000	1 lot 1:25,000 1 lot 1:25,000	1 lot 1:35,000 1 lot 1:20,000	None produced
	Average toxicity 1:27,000	Average toxicity 1:20,000	Average toxicity 1:25,000	Average toxicity 1:27,000	

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*Flasks contaminated.

†Temperature of incubation down to 32°C

‡Broth very dark, due probably to over heating of glucose.

§Broth very acid (2.2) after sterilization.

**Two lots of toxin were interchanged through error when removed from the incubator. Lot inoculated 1-26-16 was mixed with that of 2-2-16.

††This preparation is referred to in text as lot 127.

DIFFERENT PEPTONS

In the spring of 1916, we anticipated the present shortage of Witte pepton and made comparative tests with some of the domestic peptons on the market. These results are to be regarded as comparative only, but in those that were controlled by the use of Witte pepton, the differences in the toxicity, with one or two exceptions, were very significant.

A subsequent test, made with another preparation of Fairchild pepton, gave a toxicity of only 1:8000. No control test was made as we were unable to obtain Witte pepton.

TABLE 2.

COMPARATIVE TESTS OF DIFFERENT PEPTONS

TEST	NAME OF PEPTON	TOXICITY
1	Witte (control)	1:25,000
	Leitz	1:5,000
	Eimer and Amend	1:1,000
2	Witte (control)	1:25,000
	Squibb	1:5,000
	Research no. 2	1:15,000
3	Witte (control)	1:25,000
	Fairchild	1:20,000
	Research no. 2	1:20,000
4	Witte (control)	1:30,000
	Parke, Davis and Company	1:8,000

MARTIN PEPTON

Sporadic attempts at using Martin pepton broth were made early in the year 1916 with indifferent results. Nothing more was done until January, 1917, with regard to the adoption of Martin broth for tetanus toxin as used at the Pasteur Institute. The entire absence of Witte pepton at this time prevented us from making control tests with the usual Witte pepton glucose veal broth.

The highest average for this period, i.e., in the year 1917, occurred in the second quarter, when the average toxicity was 1:17,000. The first and third quarters gave respectively the average toxicity of 1:10,000 and 1:9000 while the fourth quarter gave the lowest average toxicity of 1:5,000.

Three different strains of *Clostridium tetani* were used for a comparative test (table 4). The strain designated "Research" is the culture used in the routine production of tetanus toxin at the Bureau of Laboratories. This strain was obtained about ten years ago from the New York State Laboratory at Albany but its origin and date of isolation are not known. Unless otherwise stated this was the strain used in our tests. The Goadby strain was obtained from Dr. MacConkey, Lister Institute, England, in 1916, and had been isolated from a war case by Dr. Goadby in 1915. The Pasteur strain was the one formerly in use at the Pasteur Institute, Paris, for the production of tetanus toxin. That this strain did not produce so potent a toxin (1:25,000) as the Research and Goadby strains (1:35,000) was somewhat

TABLE 3.

MARTIN PEPTON VEAL BROTH

	1917	1918
First quarter	2 lots 1:15,000 1 lot Below 1:5,000 Average toxicity 1:10,000	2 lots below 1:5,000 Average toxicity below 1:5,000
Second quarter	1 lot 1:25,000 1 lot 1:10,000 Average toxicity 1:17,000	1 lot below 1:5,000 Average toxicity below 1:5,000
Third quarter	1 lot 1:15,000 2 lots 1:8,000 1 lot 1:5,000 Average toxicity 1:9,000	None produced
Fourth quarter	1 lot 1:15,000 2 lots 1:8,000 3 lots 1:5,000 3 lots Below 1:5,000 Average toxicity 1:5,000	None produced

surprising as it had been kept on a medium made with Martin pepton for years, whereas the Goadby and Research strains had been accustomed to this medium for several months only.

That Martin pepton broth as made by us is capable occasionally of giving potent toxin, is evidenced by the results obtained in experiment 1 (table 4) and in experiment 4 (table 5.) The other preparations of toxin broth were made with the same precautions and care but, as is shown, with far different results. When it was apparent that equal parts of Martin pepton and veal

TABLE 4

COMPARATIVE TESTS OF DIFFERENT STRAINS IN MARTIN PEPTON VEAL BROTH

	Strains	Toxicity
Experiment 1. December 23, 1916.........................	Research	1:35,000
	Goadby	1:35,000
	Pasteur	1:25,000
Experiment 2. May, 19, 1917.............................	Research	1:10,000
	Goadby	1:15,000
Experiment 3. December 15, 1917.........................	Research	1:8,000
	Pasteur	1:8,000
	Pasteur	1:8,000

infusion were not giving satisfactory toxin, tests were made with broth prepared by using more concentrated Martin pepton solution with ordinary veal infusion (experiment 4, table 5); also by using the usual amount of Martin pepton solution with a more highly concentrated veal infusion. In neither case was the result so satisfactory as with the usual preparation. Subsequent broths were prepared according to the original method, that is to say using equal parts of infusion and pepton, but the toxin was far too low in potency to be used.

Word was received in January, 1918, with regard to the modification of Martin pepton broth used with favorable results for tetanus toxin at the Pasteur Institute. The variation between this procedure and the one we were using lay in the concentration of the pepton solution, in the shortened incubation period and also in the absence of glucose. A preparation of toxin broth (see experiment 6, table 6), was made according to this modification, one-half of the broth having one per cent glucose added to it while the other half was prepared without any sugar. After seven days incubation instead of the usual period of fifteen days, the cultures were filtered and tested. The results of the animal tests were most disappointing as pigs inoculated with one c.c. of a dilution of 1:5,000 from each preparation showed an absence of tetanic symptoms. In his article on the preparation of this pepton, Martin advises that not less than five stomachs should be used owing to the great variation of the different stomachs. We are accustomed to make about fifteen liters of pepton solution for one preparation of toxin broth. This amount requires seven or eight stomachs according to the size. At the Pasteur Institute, marmites or large casseroles holding not less than 60 liters are employed for the digestion of the pigs' stomachs.

TABLE 5

COMPARISON OF DIFFERENT METHODS FOR THE PREPARATION OF MARTIN PEPTON VEAL BROTH

	MEDIA	STRAIN	TOXICITY
Experiment 4. June 13, 1917......	*Control broth* 1 part Martin pepton solution (200 grams of minced stomach to 1000 cc. H_2O) 1 part veal infusion (500 grams of veal to 100 cc. H_2O)	Research	1 :25,000
	Experimental broth 1 part of Martin pepton solution 1 part of veal infusion (500 grams of veal to 500 cc. H_2O)	Research	1 :8,000
	Experimental broth 1 part of Martin pepton solution (400 grams of stomach to 1000 cc. H_2O) 1 part of veal infusion	Research	Below 1 :5,000
Experiment 5. October 10, 1917...	*Control broth* 1 part Martin pepton 1 part of veal infusion	Research	1 :5,000
	Experimental broth 1 part of Martin pepton solution 2 parts of veal infusion	Research	1 :8,000
	Experimental broth 1 part of Martin pepton solution (200 grams stomach to 1500 cc. H_2O) 1 part of veal infusion	Research	1 :5,000
Experiment 6. April 2, 1918......	1 part of Martin pepton solution (300 grams of stomach instead of usual 200 grams to 1000 cc. H_2O)	Research	
	1 part of veal infusion To the above mixture of pepton and veal infusion 1 per cent glucose was added	Research	Below 1 :5,000
	1 part of Martin pepton (300 grams of stomach to 1000 cc. H_2O) 1 part of veal infusion. No glucose	Research	Below 1 :5,000

TABLE 6

BERNA PEPTON

	1918	1919	1920
First quarter	None produced	1 lot 1:40,000 1 lot 1:35,000 1 lot 1:30,000 1 lot 1:25,000 1 lot 1:20,000 5 lots 1:15,000 4 lots 1:10,000 4 lots 1:8,000 1 lot 1:4,000 Average toxicity 1:15,000	1 lot 1:35,000 1 lot 1:30,000 1 lot 1:15,000 1 lot 1:10,000 Average toxicity 1:22,000
Second quarter ...	1 lot 1:100,000 1 lot 1: 45,000 Average toxicity 1:72,000	1 lot 1:40,000 4 lots 1:35,000 1 lot 1:30,000 4 lots 1:25,000 2 lots 1:15,000 1 lot 1:10,000 3 lots 1: 5,000* 1 lot 1: 5,000 Average toxicity 1:21,000 Excluding* average toxicity 1:25,000	1 lot 1:25,000 1 lot 1:20,000 1 lot 1:5,000 Average toxicity 1:16,000
Third quarter	2 lots 1:8,000 2 lots 1:5,000 Average toxicity 1:6,500	2 lots 1:60,000 Average toxicity 1:60,000	1 lot 1:20,000 2 lots 1:12,000 Average toxicity 1:14,000
Fourth quarter ...	1 lot 1:60,000 1 lot 1:45,000 1 lot 1:35,000 1 lot 1:25,000 1 lot 1:12,000 1 lot 1: 7,000 Average toxicity 1:30,000	1 lot 1:30,000 1 lot 1:20,000 1 lot 1:15,000 Average toxicity 1:21,000	Production for this quarter not completed

*These three lots were grown at too high temperature, i.e., 42°C.

BERNA PEPTON

About this time our attention was called by Dr. Noble of the New York State Laboratory to a pepton put on the market by the Swiss Vaccine and Serum Company of Berne, Switzerland. The statement that this pepton was made according to Witte's recipe was received with some skepticism but the thought did occur to us that this might be a means of getting Witte pepton into the Allied countries.

A small amount of broth (about 15 liters), was made up according to our usual method using the "Berna" pepton instead of the Witte. So interested were we in the result, that a small amount was withdrawn from one of the flasks, filtered and tested on the sixth day of growth. The minimum lethal dose was found to be over 1:10,000 after even this short incubation period. At the end of fifteen days the rest of the cultures were filtered and tested. The pig which received one c.c. of a dilution of 1:45,000 died of tetanus on the fourth day. A second preparation of toxin broth made with this pepton yielded a toxin of 1:100,000 in potency.

Broth made with Berna pepton has been used at the Bureau of Laboratories since May, 1918, to the present time with favorable results for the production of tetanus toxin (see table 6). The variations in the quarterly averages were greater than when Witte pepton was employed.

The average toxicities as given in this table showed again that the seasonal factor was of no importance in the production of potent toxins. The second quarter in the years 1918, 1919 and 1920 gave averages of 1:72,000, 1:21,000 and 1:16,000 respectively. The third quarter of these same years showed still greater differences. The average toxicity in this period of 1918 was 1:6,500. In the year 1919 this quarter gave a high average toxicity of 1:60,000 and the same period in the year 1910 showed an average toxicity of 1:14,000.

SUMMARY

In going over the above results, it will be seen that Witte pepton has been an important factor in the production of a fairly constant potent tetanus toxin. The indications are that the Berna pepton is a satisfactory substitute for Witte since the latter is no longer available.

It would seem either that the Swiss Serum and Vaccine Company was justified in its claim that Berna pepton was made according to Witte formula or that Berna pepton was in reality Witte pepton since only when using these two peptons did we obtain a toxicity of 1:100,000.

It was a great disappointment that Martin pepton broth as made by us proved so unreliable. It is just possible that one of the reasons for the better results with this broth at the Pasteur Institute, is due to the fact that a larger number of stomachs are used for the preparation of the pepton than we can handle at one time at the Bureau of Laboratories.

For bibliography see the original article.

TETANUS TOXIN ANTITOXIN MIXTURE FOR ACTIVE IMMUNIZATION IN GUINEA PIGS

By Edwin J. Banzhaf and Chas. K. Greenwald

During October, 1920, tetanus toxin, four months old and testing 4000 M.L.D. per cc. was selected for the preparation of toxin-antitoxin mixtures. These preparations contained respectively 5.5, 6, 6.5 and 6.75 units to each cc. of toxin. Of these mixtures the first three caused acute death in guinea pigs when five cc. was given. The latter two which correspond to about 60 per cent. and 70 per cent. more antitoxin than theoretically required to neutralize all the toxin, caused stiffness in guinea pigs with recovery when five cc. was given.

A mixture was then prepared containing 90% over the theoretical required amount, five cc. of which caused no stiffness in guinea pigs. This over neutralized mixture was used for active immunization in guinea pigs as shown in the following table.

RESULTS OF A SINGLE IMMUNIZING DOSE OF THE OVER NEUTRALIZED MIXTURE OF TETANUS TOXIN-ANTITOXIN

Date Injected October 29, 1920	Dose	9 Weeks Later Injected with 1.5 M.L.D. of Tetanus Toxin	Results
	1/10 cc.	1.5 M.L.D.	Dead 2 days
	1/10 cc.	1.5 M.L.D.	Dead 4 days
	1/10 cc.	1.5 M.L.D.	Dead 6 days
	1/10 cc.	1.5 M.L.D.	Dead 5 days
	1/10 cc.	1.5 M.L.D.	Lived
	1/4 cc.	1.5 M.L.D.	Lived
	1/4 cc.	1.5 M.L.D.	Lived
	1/2 cc.	1.5 M.L.D.	Lived
	1/2 cc.	1.5 M.L.D.	Lived
	5 cc.	1.5 M.L.D.	Lived
		Control 1 M.L.D.	Dead 4 days

Definite evidences of active immunization are shown when only one injection is given and when the mixture is 90% overneutralized. The foregoing preparations were stored in the refrigerator and two years later, on retesting, the mixture containing 5.5 units antitoxin (35% excess) was found to cause slight stiffness in guinea pigs when five cc. was injected.

Guinea pigs receiving a single injection of 1/10 c.c. of above mixture, withstood five M.L.D. of tetanus toxin three months later, without any signs of stiffness.

It is interesting to note that as little as 1/25 of a fatal dose of tetanus toxin in a guinea pig will cause definite stiffness.

MICROBAL STUDIES ON ACUTE RESPIRATORY INFECTION WITH ESPECIAL CONSIDERATION OF IMMUNOLOGICAL TYPES[1]

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William H. Park, Anna W. Williams and Charles Krumwiede

Introduction

Outline of work planned, discussion of results and general conclusion

It was decided at a conference of the members of the Influenza Commission[2] and the Research Laboratory workers that the problems[3] to be investigated in New York City should be the following:

1. A study of the microörganisms of the upper respiratory tract in "health," in "common colds" and in "influenza." This investigation should include the diligent search for an epidemic microbal strain in influenza cases. Both filtrable and unfiltrable microbes were to be considered.
2. A continuation of our studies on hemoglobinophilic bacilli (group of the influenza bacilli) for the purpose of establishing the relationship of the influenza bacillus to both pandemic and sporadic cases of influenza.
3. A study of the permanence of type characteristics of influenza bacilli in persons after recovery from respiratory infections. It was considered of the utmost importance to establish as accurately as possible the stability of types.
4. A study of the incidence of common colds, "influenza," and pneumonia, following the controlled use of a vaccine made up of different types of bacteria claimed to be the cause of acute respiratory diseases.

In carrying out this work, a large part of the material for the first two problems was collected from employees of the Metropolitan Life Insurance Company who volunteered their aid. The rest of the material was collected from laboratory workers and from cases of "influenza" in the hospital for contagious diseases. The preventive value of the vaccine was tested upon 1600 of the employees of the Metropolitan Life Insurance Company.

In arranging the work for the first problem, the investigation of the incidence of microbes was planned to include enough normal cases so as to have for study a sufficient number of persons who would later develop acute "colds" or "influenza" in order to obtain significant comparative curves of microbal incidence beginning in pre-disease stages and continuing throughout the attack; also to note the effect, of any, of vaccination upon the subsequent microbal content of a case. Our plans were changed, however, because of an early outbreak of acute respiratory infections which were diagnosed shortly after as influenza. In our opinion there is considerable doubt as to the influenzal nature of many of these cases. Notwithstanding our large force of workers, we were unable to investigate these and at the same time study normal cases as fully as we had planned. We were afraid to postpone study of the new cases as the outbreak might stop and we should then have had no acute influenza cases for study. We concentrated, therefore, on a mass of "influenza" cases, hoping, in spite of our previous failure, to demonstrate a common microbal strain whether filtrable or non-filtrable.

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[1] A series of papers from the Research Laboratory, Department of Health, New York City, and one from the New York Hospital.

[2] The members of the commission are Drs. M. J. Rosenau, G. W. McCoy, Lee Frankel, A. S. Knight, E. O. Jordan, W. H. Frost and W. H. Park.

[3] The studies are a part of the investigation on the etiology and the prevention of the acute respiratory diseases being carried out in Boston, Chicago, Washington and New York under a grant from the Influenza Commission Fund of the Metropolitan Life Insurance Company.

In the first paper we attempted to show the value of different technical methods in the demonstration of the different species of microörganisms from the upper respiratory tract and to give the average comparative incidence of groups of microörganisms demonstrated by these methods in health and disease. We lay stress on the methods of obtaining and handling the material.

In the succeeding papers in this number of the Journal and in other papers which will appear later, are reported the studies made on the isolations from colonies of the various species appearing on the original plate cultures made directly from the case or from the inoculated mice. The purpose was the search for a common "outbreak" or epidemic strain among the cultures isolated.

In two additional papers in this number are given respectively: A brief history of two cases of accidental infection with the influenza bacillus and the persistence of type characteristics in the inoculated persons; The results of vaccines on the prophylaxis of respiratory inflammations.

Discussion

The chief object of all of these investigations was to demonstrate a common "epidemic" or "outbreak" strain among the groups of microbes claimed to have caused epidemics. As much as we were able, we employed methods that might best demonstrate these groups. We continued to bear in mind, however, that the methods we used might also demonstrate in small numbers hitherto undescribed organisms that might have an etiological relationship to these infections. In spite of this, we decided to study closely only the more dominant types that were demonstrated by our procedure, simply recording the minority groups as determined by colony appearances and the examination of stained specimens from these colonies. We recognized that our methods of collecting material did not take into account the possibility of the primary exciting factor growing chiefly beneath the surface of the mucous membrane. We believe we are justified in assuming the probability that such a specific microbe would predominate or at least be prevalent upon the surface of a localized area of mucous membrane during some part of the early stages of the infection. If this microbe were an aerobe capable of growing on the culture media employed, selected fishings from ten colonies of the dominant types appearing on these media should give a majority of cultures obtained from the epidemic cases having no fundamental differences.

The results of our studies indicate that of the different groups of microörganisms isolated by our procedure, all had the peculiarity that each group was an assemblage of many types. This was equally true for the pneumococcus, the influenza bacillus, the green-producing streptococci and the hemolytic streptococci. We obtained no evidence of the existence of a common filtrable organism. We accepted as the most delicate test of identity of types in strains the presence of specific agglutinable and agglutinogenic substances as determined by direct agglutination and agglutinin absorption methods. Biochemical characteristics are insufficient for such a determination; at most they divide a species only into the broader groups. In our isolation work, we are aware that the number of colony fishings may not have been enough in every group of bacteria to rule out a common strain. In a study of case incidence alone, some groups may be ruled out as implicated in the epidemic; such as staphylococci, Gram-negative cocci and hemolytic streptococci. More work could be done on pneumococci, green streptococci, indifferent streptococci and some of the minority groups with a high case incidence. The question of the relationship between the bacterial types used in vaccination and the microbal strains obtained from the throats of the vaccinated remained mostly unanswered. The specific strains of organism used in the vaccines were not found to any extent in either normals or the diseased. It is a curious fact that in all but one instance the fixed types of pneumococci, as found in eleven of all the cases examined, occurred among the unvaccinated influenza cases. This loses significance, however, in the light of the fact that only 6 of our cultivated influenza cases had received vaccine and 42 had not.

The evidence of immunological response to the vaccine was, as might be expected from the above findings, apparent only in the lessened incidence of pneumonia. The percentage of colds was as great among the vaccinated as among the unvaccinated. The pneumonia incidence was much less. The greater multiplicity of types of microbes believed to be capable of exciting common colds over those usually exciting pneumonia, is possibly the explanation of the apparent uselessness of the vaccines employed in this series in preventing minor respiratory infections while apparently affording considerable protection against pneumonia.

STUDIES ON ACUTE RESPIRATORY INFECTIONS

Methods of Demonstrating Microörganisms, Including "Filtrable Viruses," from Upper Respiratory Tract in "Health," in "Common Colds" and in "Influenza" with the Object of Discovering "Common Strains"

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Anna W. Williams, Mary Nevin and Caroline R. Gurley
Assisted by Alice Mann, Helena Hussey and Florence Bittman

Choice of Methods

From the standpoint of arranging methods, we divided the microörganisms for which claims had been made into two general groups, namely, aerobic and anaerobic microbes, subdividing each into filtrable and non-filtrable microbes. As the most vigorous claims had been made for certain aerobic non-filtrable microbes and for anaerobic filtrable ones, we arranged our methods with special reference to these. We divided the aerobic non-filtrable organisms further into four general groups; namely, Gram-positive cocci, Gram-negative cocci, hemoglobinophilic bacilli and "others." We concluded that one of the nearest approaches to a standard method for demonstrating filtrable organisms from the upper respiratory tract was that reported by Foster (chiefly Noguchi technic). For this reason and because we wished to corroborate his work, we decided to use his method as well as modifications of it.

In regard to demonstrating the aerobic non-filtrable microbes from the mucous membranes of the upper respiratory tract, although no comprehensive method of procedure was found that was described fully enough to be used, we decided that certain culture media with methods of inoculating them might be considered standard. Among these were Brown's technic for the demonstration of Gram-positive streptococci and Avery's medium for hemoglobinophilic bacilli. Other media which were used are described in the original article. The technic for the collection of material and for first cultures is described in the original article.

Results

1. From aerobic cultures of non-filtrable material.

Although the cultural results were influenced greatly by the area from which the material was obtained, the effect of the different kinds of media was equally marked. Thus on our vitamin medium we had abundant growths of all groups, so abundant, that in many cases we had overgrowths. On Avery's oleate agar, as was to be expected with an inhibiting medium, chiefly influenza bacilli and certain Gram-negative cocci and other Gram-negative bacilli grew. It is interesting that most strains of meningococci do not grow well on this medium, while most strains of gonococci and *B. pertussis* do. On veal blood-drop plates, by our procedure, the proportion and size of influenza bacilli colonies were less, while in Brown blood pour plates the influenza group and all other groups, except the Gram-positive cocci, appeared usually in still smaller numbers. This was due, partly, to our manner of inoculating the material but in a series of cases where we put the swab from the case directly into the diluent (pneumococcus broth) and inoculated equal proportions of broth in each medium, our results were practically the same. The copy of one of our primary case records indicates these differences.

2. From anaerobic cultures of non-filtrable washings.
No filtrable organisms were found.

3. From anaerobic cultures of filtered washings.
In six out of the forty cases cultured in this manner, anaerobic organisms were isolated. Two cases yielded a Gram-positive non-hemolytic streptococcus, one other case a diphtheroid; two cases a Gram-negative coccus and one a case a small Gram-positive coccus. In only one instance was the growth found in more than one of the inoculated tubes and in this instance only two showed evidence of growth. The controls remained sterile. Meat medium cultures of all of these organisms were filtered through Mandler filters. No growth occured in any of the cultured filtrates.

No further work was done with these cultures because they seemed to be of no etiologic significance. On the contrary, they were regarded as contaminants, although all of the control tubes (2 meat and 3 horse serum glucose agar) in each case remained sterile.

4. From anaerobic cultures of filtered washings. (Noguchi techinc chiefly).

Growths were obtained in the anaerobic cultures of filtrates from 4 out of 10 consecutive cases of influenza. In all of these cultures, a minute coccoid organism, differing somewhat morphologically in two of the cases, was found. These were all obtained from the filtrate of the nasopharyngeal washings but they could not be carried on for more than one culture generation. No animal inoculations were attempted.

Of 23 early rhinitis cases, one showed an anaerobic coccus which died out in the third culture generation; one other case, a coccus, probably anaerobic, which died out in the second culture generation. In 20 normal washings no organisms were found.

From the filtrates of 7 early "cold" cases of a type similar to that described by Foster and from three typical influenza cases, we were unable to infect 45 human volunteers, 20 of whom were students less than 30 years of age. In the method of collecting the material and doing the inoculations Foster's technic was followed as closely as possible.

Comparative Incidence of Microorganisms from Nasopharynx as Recorded in Our Original Records

In trying to indicate the changes which we found in "normals," "colds" and "influenza" in the incidence of the group of microörganisms according to the chart headings, we obtained from our primary cultures the common incidence in each group by estimating the average percentages for each case on all the culture media used, adding these percentages in each individual group and dividing the sum by the total number of cases in that group. The following chart gives this common incidence in percentage for each group of cases.

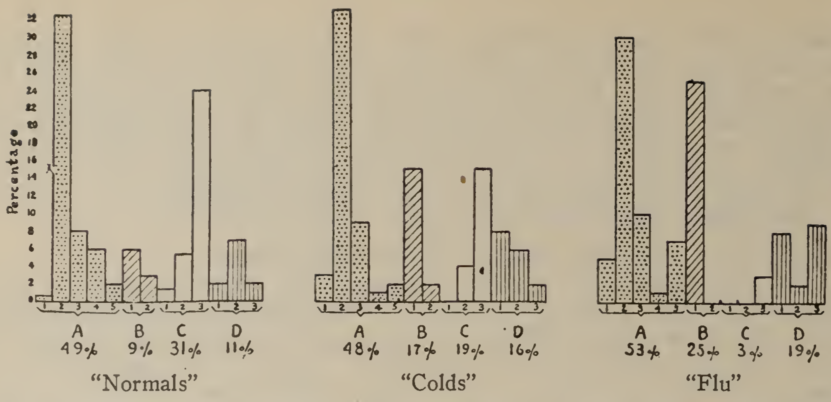

Chart Showing Comparative Incidence of Microörganisms by Groups (Reprinted by courtesy of Journal of Immunology)

Explanation of Chart

A = *Gram-positive cocci.*
  1. "Hemolytic streptococci" (Smith and Brown's beta type).
  2. "Green producing streptococci" (Smith and Brown's alpha type).
  3. Pneumococcus group.
  4. Staphylococcus group.
  5. "Indifferent streptococci" (Brown's gamma type).

B = *Hemoglobinophilic bacilli.*
  1. "Typical."
  2. "Atypical."

C = *Gram-negative cocci.*
  1. Meningococcus group.
  2. Micrococcus catarrhalis group.
  3. *M. flavus, M. siccus* and others.

D = *Other microorganisms.*
  1. "Large Gram-negative bacilli."
  2. *Bacillus mucosus* group.
  3. "Others."

Interpretation of Chart

In the first place it should be remembered that the chart and the table give no indication of the amount of growth. That was recorded on the original sheets and may be summed up as follows: From the nasopharynx of the "normal cases," on the whole a very scant growth was obtained. In the dilutions we employed, we often obtained no growths on our Brown plates, in fact, from some cases there was practically no growth on any plates, except on the vitamin-blood-agar and the oleate, where the growth was a scanty one. In the "colds," the growth on the whole was very abundant, though in some of the cases obtained in the first hours of the disease, the amount was scanty. In all of the influenza cases, the growth from the nasopharynx was very abundant.

In studying the comparative incidence in the types of clinical cases of the four groups of microörganisms as shown by the chart, we note that there are few differences if the groups are considered as a whole. We see simply that the Gram-negative cocci group drops somewhat in the "cold" cases and markedly in the influenza cases, while the influenza bacilli and the "others" progressively increase. When we study the subgroups, however, more differences appear. But we must take into account more minute colony characteristics in order to appreciate further differences.

Thus, among the Gram-positive cocci, the "alpha type" of streptococcus (green-producing streptococci) predominate in all three kinds of cases. But in the "normals" and "colds," this type varied in general colony characteristics in the different cases while in practically every influenza case the majority of green-producing colonies seemed to be of one type and of a type that had not appeared before, at least in any numbers. We labelled these colonies "minute green" and these are the colonies from which isolations were made for further study and comparison with the fishings of green-producing colonies which we had made from the "normals" and "colds." (See paper III of this series.)

The "beta type" of streptococcus shows a small increase when compared with group incidence, although in disease the increase over its appearance in normals is marked. It is significant that out of 12 cases of colds in which hemolytic streptococci appeared in the cultures from the nasopharynx, 7 of the cases began as tonsillitis. The further study of the beta streptococci from this series of cases is to be reported later.

The pneumococci show a very slight increase in "colds" over the "normals" and in "flu" over the "colds." The further study of the strains is given in Paper II of this series. No common type was found. It is interesting that of the few fixed types found in the influenza cases, all occurred in the ten cases not inoculated with our vaccine (see Povitsky paper), but, as only 6 of the 48 cases in this series had been inoculated with the vaccine, this observation has little, if any, significance.

Since the percentage of staphylococci were infrequently demonstrated from the nasopharynx in disease, we decided to discontinue the study of the individual fishings.

Although there was a marked increase of the "indifferent streptococci" (Smith and Brown's gamma type) in "colds" and "influenza," over the percentage usually found in "normals," there were very few in the individual cases. However the increase occurring in "flu" case incidence indicates that a minority curve might be found that may be of significance.

That among these colonies diagnosed originally as "indifferent," that is, producing no changes on standard blood-agar-plates, some may be found on further study to be alpha streptococci type, is possible. We fished a number of these colonies from many cases and studied primary cultures in a general way hoping to be able to classify them further, but we had to discontinue their study because of the emphasis we were placing on majority incidence.

In regard to the hemoglobinophilic bacilli the most interesting point brought out in the chart, is that the atypical influenza bacilli (see Povitzky's paper) occur less commonly in the "cold" cases than in the "normals" and drop out entirely in the primary cultures from the influenza cases which were studied. This makes the increase in the typical influenza bacilli in the "influenza" cases very marked. The case incidence is 92 per cent. Of course, we show (Povitzky's paper) that there is no one serologic type in this series of cases, thus corroborating our earlier work.

In some of the early "cold" cases, we found large numbers of *M. catarrhalis*. Chiefly for this reason and partly because claims had been made for Gram-negative cocci as an etiological factor, we started to study individual fishings of this group. We studied them for only a short time, however, owing to the fact that neither they nor practically any kind of Gram-negative coccus appeared in our "influenza" cases.

Although types of Gram-negative cocci may have a relationship to certain cases of "common colds," judging from the comparative incidence none had anything to do with the influenza cases in this series.

Among the "others" we recorded and fished colonies of large Gram-negative bacilli showing similar characteristics. These bacilli occurred in increased abundance over normals in "colds" and "influenza" cases with a corresponding increase in case incidence. We made many fishings from this type of bacillus but were unable to finish our studies on it. We have concluded that it may be an important factor in "common colds."

B. mucosus, we recorded because of its relatively abundant growths in certain cases in the "normals" and "colds" but we made no study of individual fishings. It dropped almost out of sight in the influenza cases. The last column in the Chart in the groups of "normals" and "colds" is a record of different organisms from individual cases, some Gram-negative bacilli, some Gram-positive bacilli which were different in the different cases. On the other hand, in the "influenza" cases, this record is due chiefly to "minute" delicate colonies appearing on our first vitamin-blood-agar plates in close proximity to colonies belonging to other groups. When we diluted the material further they dropped out. We attempted to isolate these organisms and obtained in the majority of cases a minute cocco-bacillary form of short chains and clumps. These died out quickly (we made the majority of our fishings on chocolate agar) and as they were in such great minority and we were studying majorities we discontinued their study.

CONCLUSIONS

The procedure for collecting and handling material as outlined in this paper is a more comprehensive one than has been given before for demonstrating the comparative incidence of the great groups of microörganisms that have usually been found in the majority of acute upper respiratory infections. Therefore, the colony fishings are more likely to be representative of a dominant type, if one exists.

The procedure has also demonstrated increased incidence in mucous membrane inflammations of certain minority groups due to an increase of one type of organism which might have a relationship to acute inflammations of the upper respiratory tract.

The negative results obtained by our procedure for demonstrating filtrable microbes, we consider an indication that the positive results already reported do not bear as wide an application as their sponsors infer.

For bibliography see the original article.

A STUDY OF THE SEROLOGICAL RELATIONSHIPS OF PNEUMOCOCCI FROM THE UPPER RESPIRATORY TRACT WITH SPECIAL REFERENCE TO COMMON COLDS AND INFLUENZAL CONDITIONS

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Georgia M. Cooper, Lucy Mishulow and Ninette E. Blanc

This study forms a part of a general investigation by the Bureau of Laboratories and the Influenza Commission of the Metropolitan Life Insurance Company of the flora of the respiratory tract in normal and pathological conditions. The purpose of this section of the work was to determine whether any one variety of pneumococcus would be encountered more frequently in epidemic inflammatory conditions resembling influenza than in those of the nature of ordinary colds and in the normal controls. If, in a wave of common colds or influnezal-like inflammations, a dominant variety could be demonstrated in the majority of cases, it would suggest strongly that the pneumococcus strain in question was the primary causative agent of the outbreak in which it was prevalent. The presence of a single type of a pathogenic variety in the individual case may indicate, however, that it is either the primary agent or one of the secondary invading bacteria, or it may be only an accidental parasitic variety. The problem was, therefore, to determine the similarities or dissimilarities among the various strains isolated from the nasopharynx of normal individuals and of cases of rhinitis, pharyngitis, influenza and broncho-pneumonia. In all instances the nasopharyngeal material examined was the same as that from which the other species of organisms were isolated by the different workers engaged in this general investigation. The methods employed for the study of the similarities and dissimilarities of the pneumococcus strains were those of agglutination and agglutinin absorption.

The study of the strains was based on the facts already known concerning the differentiation of the pneumococci. These facts may be briefly reviewed as follows. Park and Williams classified *Streptococcus mucosus* as a definite variety of pneumococcus, which variety is now known as *Pneumococcus mucosus* or Pneumococcus, type III. Collins, resorting to agglutination and agglutinin absorption with monovalent rabbit serums, found specific relationships among members of this mucosus group and group relationships with other varieties of pneumococci. Cole and Hanes also demonstrated marked cross agglutination among members of this group. Neufeld and Händel also found specific types of pneumococci, which types were influenced only by their own antiserums. These observations were confirmed and extended by Cole and by Dochez and Gillespie who showed that many pneumococci could be placed in two distinct serological types which they termed type I and type II. They defined type 3 as a distinct morphological variety and placed the strains which could not be classified with the above types in a heterologous group of strains termed group IV.

Pneumococci giving a partial or delayed agglutination with type II serums were later defined by Avery as atypical type II or subgroup II strains. According to him, type II serum protected mice against lethal doses of the majority of these strains. He classified ten strains in two distinct groups, subgroup A and subgroup B and a third heterologous group subgroup X. Subgroup A removed from type II serum the agglutinins for other members of that group but not for subgroup B. The subgroup B acted vice versa. However, neither of these removed the specific agglutinins for type II strains. With monovalent rabbit serums for the members of the subgroups, he found that subgroup A strains gave marked agglutination with antiserums for members of that subgroup; that subgroup B strains agglutinated with subgroup B antiserums but there was no cross reaction between these subgroups. Typical type II strains gave no reaction with the antiserum of either subgroup. Stillman following the methods of Avery classified 204 strains in twelve subgroups, the subgroups II-A and II-B of Avery and ten others, subgroups II-C, D, E, F, G, H, J, K, L, and M. The attempt to demonstrate definite subgroups among the strains provisionally classed as group IV, is considered later.

The literature on the incidence of the types of pneumococci in pneumonia is very extensive. The incidence of the different types in normal individuals, including healthy contact carriers, has also been studied in detail but there is little information available on the occurrence of types in other conditions. Park and Williams in 1905 investigated the pneumococci isolated from inflammations of the upper respiratory tract. At that time the separation of types had not been completed and correlation of their groups with those now recognized is not possible. Valentine investigated the incidence of types of pneumococci in common colds with a view to their being a possible source of contagion for lobar pneumonia. She found pneumococci in forty-three out of sixty-five cases of common colds. The incidence of types was as follows: Group I, two cases; group II, two cases; group III, four cases; and group IV, thirty-five cases. Clough published a study in which a few conditions other than pneumonias were included, but for the most part the conditions investigated by her do not coincide with those studied by us. In five cases of chronic bronchitis she found three type IV strains and two strains classed as miscellaneous. In two acute bronchitis cases, one strain was an atypical type II and the other a type IV.

Of 138 cases cultured by us, pneumococci were found by the methods employed, to be present in fifty-three. The criteria adopted by us for identification of an organism as a pneumococcus were typical morphology and bile solubility. All of the cultures fermented inulin, but a strain would not have been discarded had the ability to ferment this carbohydrate been lacking. In one instance a culture was lost before typing was completed; in five the pneumococci were not isolated in pure culture because the plates were overgrown by *B. subtilis* or *B. proteus*. In these instances, it was inferred that pneumococci were present from the discovery of typical Gram-positive encapsulated diplococci in the smears and from the bile solubility of the cocci obtained from the peritoneal washings of the injected mice.

Strains were obtained, therefore, from forty-seven cases. These strains were then tested with serums of the three types. For the identification of the fixed types, undiluted antipneumococcus horse sera were employed and the tests were incubated at 45°C. Of the forty-seven strains, nine were found to belong to the fixed types as shown by the prompt reaction with one or the other serum. These nine were distributed as follows: Type 1 pneumococci were isolated from two normal cases and one influenza; type 2 from two influenza cases; type 3 from four cases—one chronic rhinitis, one acute rhinitis, one pharyngitis and one influenza complicated by broncho-pneumonia. From the last case, a pneumococcus of subgroup IIB was isolated as well as pneumococcus type III. These results are included in the summaries (tables 1 and 2) and are discussed later in connection with the findings from further study of the other strains which did not fall into the fixed types.

The thirty-eight remaining cultures were further tested with type serums. The technic employed is given in the original article.

Every effort was made to obtain the highest possible titer. In some instances, a titer of 1:200 was the highest obtainable. Serums less potent than this were not used.

The result of the agglutination tests with these serums are given in a table in the original article.

In the instances where cross agglutination was marked, the specificity of the reactions was investigated by agglutinin absorption tests. The technic is given in the original article.

To summarize: Among the strains not belonging to the fixed types but which were covered by the monovalent serums available, were found six small groups and thirteen strains unrelated one to the other or to the groups just mentioned. This gives a total of nineteen types. Of these, one group and two separate strains belonged to the subgroup II classification. Five groups and eleven unrelated strains were provisionally placed in group IV. Of these, two groups and four unrelated strains belonged among those which gave slight agglutination with type II serums but were not further investigated as to whether they were true subgroups of type II. The strains of another group and one unrelated strain gave slight agglutination with all three type serums. Finally two groups and six unrelated strains comprised those which did not agglutinate at all with the fixed type serums.

There were, in addition, nine strains which were not similar to any of the strains for which there were antiserums. As no antiserums were prepared for these nine, the relationships among them could not be investigated. It is reasonable to suppose that further tests with these strains would yield results similar to those found among the strains for which antiserums were produced.

The results from this part of the investigation are collected in table 1 and are summarized with reference to type and pathological condition in table 2. From these data, the prevalence of a particular type of pneumococcus in any of the conditions studied is not indicated.

In addition to the classification of strains from different cases, a brief study was made of strains isolated from the same case to determine whether several varieties of pneumococci would be found in the naso-pharynx. Colonies morphologically different as far as possible were chosen, varieties of contour, size and color being noted. The agglutination of the strains from individual cases with the antiserum homologous to one strain from each case are given in table 3. In other cases, a serum was considered homologous even if not actually prepared with a strain from a case, if the absorption of the serum by a strain of the case in question was complete. The results with serums considered homologous on the basis of absorption are given in table 4. In but one case was there encountered more than one variety of pneumococcus. In all cases where a subsequent examination of an individual was made, the same variety was isolated again. The instance in which two varieties were encountered has been noted.

For the most part, the fishings considered in tables 3 and 4 were made from a single collection of material. In a few cases, material for cultures was

TABLE 1

TABULAR SUMMARY—VARIETY OF PNEUMOCOCCUS FROM CASES IN RELATION TO CONDITION

NORMAL		CHRONIC RHINITIS		ACUTE RHINITIS		PHARYNGITIS	
Case number	Type	Case number	Type	Case number	Type	Case number	Type
36	Type I	83	Type III	49	Type III	125	Type III
41	Type I	23	Group IV (5)	17	Group IV^{11} (3)	107	Subgroup II B (1)
57	Group IV^{11} (het)	52	Group IV (6)	82	Group IV^{11} (het)	131	Group IV (?)
72	Group IV^{11} (het)			89	Group IV^{11} (het)		
34	Group IV* (?)			94	Group IV^{11} (het)		
69	Group IV* (?)			39	Group IV* (?)		
76	Group IV* (4)			65	Group IV* (?)		
2	Group IV (het)			78	Group IV* (?)		
71	Group IV (het)			99	Group IV* (?)		
				38	Group IV (5)		
				130	Group IV (6)		

RHINITIS AND PHARYNGITIS		INFLUENZA		INFLUENZA AND PNEUMONIA		PNEUMONIA	
Case number	Type	Case number	Type	Case number	Type	Case number	Type
18	Group IV* (?)	164	Type I	157	Type III and Subgroup II B (1)	86	Group IV (het)
169	Group IV (6)	144	Type II	168	Subgroup II A		
		146	Type II	161	Gruop IV^{11} (3)		
		90	Subrgoup II H				
		135	Subgroup II B (1)				
		166	Subgroup II B (1)				
		134	Group IV^{11} (2)				
		158	Group IV^{11} (2)				
		154	Group IV* (4)				
		159	Group IV* (?)				
		165	Group IV* (het)				
		156	Group IV (het)				
		160	Group IV (het)				
		163	Group IV (het)				

IV^{11} Strains which gave some agglutination with type II serum.

IV* Strains which gave some agglutination with all three type serums.

Figure in parentheses is an arbitrary symbol to note the groups encountered, based on agglutinin absorption.

(Het) Heteroganous on serum reactions.

(?) No homologous serums available, did not show relationships.

taken a second or third time after intervals as recorded in the tables. Attention must be called to the fact that in many instances the strains of pneumococci were obtained from mice injected with the original material. That by this method only one variety was recovered from a case was not surprising. It was to be expected that the mouse would serve in a selective manner; the most virulent variety would most likely be recovered in predominance or alone. However, where pneumococci were obtained from plates inoculated directly with the material from the nasopharynx, the results were the same as after mouse passage.

The results of previous workers as regards the fermentative reactions of pneumococci do not suggest that these reactions would be of value in the differentiation. Park and Williams found some differences in mannite fermentation; about 70 per cent of their strains fermented this alcohol. All their mucosus types gave a positive reaction. Avery[2] found differences in fermentation with mannite and salicin but these differences did not correlate with the serological results.

In spite of the above data, it seemed desirable to determine the fermentative characteristics of the strains classified by us as distinct groups on the basis of agglutinin absorption. Similar groups have not been studied in this connection; the greater attention has been placed on the dominant fixed types. From one to six strains of an individual case were employed.

TABLE 2

TABULAR SUMMARY: NUMBER OF STRAINS OF EACH VARIETY IN RELATION TO THE CASE CONDITION

GROUPS	NORMAL	CHRONIC RHINITIS	ACUTE RHINITIS	PHARYNGITIS	RHINITIS AND PHARYNGITIS	INFLUENZA	INFLUENZA AND PNEUMONIA	BRONCHO-PNEUMONIA	TOTALS
Type I	2	—	—	—	—	1	—	—	3
Type II	—	—	—	—	—	2	—	—	2
Type III	—	1	1	1	—	—	1×	—	4
Subgr. II A	—	—	—	—	—	—	1	—	1
Subgr. II (B) (1) ...	—	—	—	1	—	2	1×	—	4
Subgr. II H	—	—	—	—	—	1	—	—	1
Group IV^{11} (2)	—	—	—	—	—	2	—	—	2
Group IV^{11} (3)	—	—	1	—	—	—	1	—	2
Group IV^{11} (het.)	2	—	3	—	—	—	—	—	5
Group IV* (4)	1	—	—	—	—	1	—	—	2
Group IV* (het.)	—	—	—	—	—	1	—	—	1
Group IV* (?)	2	—	4	—	1	1	—	—	8
Group IV (5)	—	1	1	—	—	—	—	—	2
Group IV (6)	—	1	1	—	1	—	—	—	3
Group IV (het.)	2	—	—	—	—	3	—	1	6
Group IV (?)	—	—	—	1	—	—	—	—	1
Total	9	3	11	3	2	14	3	1	

See Table I for explanation of symbols.

× Types isolated from the same case.

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[2] Results not published. Personal communication.

TABLE 3

AGGLUTINATION OF STRAINS FROM INDIVIDUAL CASE WITH ANTISERUM FOR ONE STRAIN FROM THE SAME CASE

CASE NUMBER	EXAMINATION NUMBER	DAYS ILL	ISOLATED FROM	SERUM USED	AGGLUTINATION TITRE OBTAINED WITH STRAINS NUMBERED							
					1	2	3	4	5	6	7	8
168	II	15(?)	m	168-V-1		500	500	500				
168	IV	22(?)	m	168-V-1	600	500		600				
166	I	3	m	166-II-I	200	100	200					
166	II	7	m	166-II-I	200	100						
166	III	13	m	166-II-I	200							
90	III	4	m	90-III-1	500	500	500	500				
158	I	2	m	158-I-1	400	300	400	400				
158	II	7	m	158-I-1	400	400	400	300				
17	I	1	m	17-I-1	400							
17	I	1	pl	17-I-1		400						
72	II	—*	m	72-II-4	500	600	600	600				
89	II	2	m	89-II-1	300							
89	III	?	m	89-II-1	300	300						
57	I	—	m	57-I-5	200	200	200	200	200	200		
94	I	1	m	94-I-5	300	300	300	300	300	300		
76	I	—	m	76-I-1	300	300	300	300	300	300		
154	I	1	m	154-I-1	400	400	400	400	400			
165	I	4	m	165-I-1	200		200					
38	II	½	m	32-II-2		400	400	400	400			
52	I	?	m	52-I-1	200	200	300	200	200			
130	I	¾	m	130-I-1	600	600	500	600	700			
2	II	—	m	2-II-1	400	500	300	400	500			
71	I	—	pl	71-I-5	400		400		400	400		
86	I	9	pl	86-I-1	300	400	300	300	300	400	300	
156	I	2	m	156-I-1	300	200	300	300		300		
160	I	2	pl (a)	160-I-1 (a)	300	200	200	200	200	200	300	200
160	I	2	pl (b)	160-I-1 (a)	200	200	200	200	200	200	200	200
160	I	2	m	160-I-1 (a)	200	200	300	300				
163	I	2	m	163-I-1	200	200	200	200				

*Symbol — = normals.

TABLE 4

AGGLUTINATION OF STRAINS FROM INDIVIDUAL CASE WITH ANTISERUM FOR STRAINS SHOWN TO BE THE SAME ON AGGLUTININ ABSORPTION

CASE NUMBER	EXAMINATION NUMBER	DAYS ILL	ISOLATED FROM	SERUM USED	AGGLUTINATION TITRE OBTAINED WITH STRAINS NUMBERED				AGGLUTINATION TITRE, SERUM STRAIN
					1	2	3	4	
135	I	1/24	m	166–II–1	300	300	200	200	300
157	II	8	m	166–II–1	300	300	300	300	300
134	I	1/6	m	158–I–1	300	300	330	300	300
161	I	1	m	17–I–1	300	400	400		300
161	III	7	m	17–I–1	300	400			300

All the strains except two, promptly and vigorously, fermented inulin, lactose, raffinose and saccharose. The two strains from case 169 showed less avidity for inulin than the other strains. These positive results are not tabulated. The strains showed considerable variation however in their ability to ferment salicin and mannite. These results are given in the original article.

The results show a complete lack of any correlation between fermentative reaction and agglutinative results. There is a marked tendency for the results to vary on different media as well as upon the same medium. A few of the strains show a greater and a more constant tendency to ferment. Variable results were obtained even with individual fishings from a single case.

On the whole, therefore, the conclusion seems justified that the ability to ferment salicin is not a fully developed characteristic. The ability to ferment mannite is even less developed. The condition is comparable to the variable avidity for certain carbohydrates or alcohols shown by members of the typhoid-paratyphoid dysentery groups.

Conclusions

The serological relationship of fifty-five strains was studied. These were isolated from the naso-pharynx of normal controls, from cases of rhinitis, and pharyngitis, also from influenza-like cases and influenza-like cases complicated by broncho-pneumonia. No single variety of pneumococcus was found to be dominant in the inflammatory conditions studied. This was as true for the influenza-like cases as for common colds. At most the only difference between the normal and the acute inflammatory condition was a slightly greater incidence in the latter of the different varieties classified as sub-groups of type II. There were no data, therefore, which indicate that the pneumococcus was the primary etiological agent in the contagious types of inflammation.

In twenty-five cases from which several strains were isolated from each individual, all the strains from the individual case were similar with one exception. In this case two varieties were found. These findings indicate that usually one variety of pneumococcus predominates in the naso-pharynx.

Some of the serological groups as well as individual strains, have been classified as members of the sub-groups of type II by the use of control strains. Other groups and individual strains seemingly should be classified in the same manner. Some groups belong apparently in group IV. The question whether strains should or should not be classified as belonging to the sub-groups of type II is not satisfactorily determinable with the criteria available.

The fermentative results with salicin and mannite were inconstant and the differences among strains showed no parallelism with the serological findings. Such differences have, therefore, no evident classificatory significance.

For bibliography see the original article.

RELATIONSHIP TO UPPER RESPIRATORY INFECTIONS OF STREPTOCOCCI PRODUCING A GREEN ZONE ON STANDARD BLOOD AGAR PLATES (SMITH AND BROWN'S ALPHA TYPE)

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Anna W. Williams, Aarlaug Unneberg, Agnes Goldman and Helena Hussey

The primary object of this study, as we have already stated in the introduction, was to look for evidence of an epidemic strain among cocci of this type.

With regard to this group of bacteria, perhaps more than any other, sweeping statements have been made concerning the relationship of strains to epidemics. This is largely due to the ubiquity and the difficulty of demonstrating clear cut proofs of such relationship and also to the ease with which deductions are made from insufficient evidence.

The most important question relating to this subject about which we have little accurate knowledge, is the question of the permanency of those characteristics which are supposed to be indicative of species, of specific pathogenicity and of related characteristics which might be used in diagnosis.

We know a little of the limits of change in *vitro* but essential changes in the animal have not yet been demonstrated with controls sufficiently free from error. Indeed, until we can employ a technic that seems now to be practically impossible in its minutia, we cannot claim to have demonstrated fundamental changes occurring in microorganisms grown in either test tube or animal.

So much has been claimed for this group of organisms, especially since the "flu" pandemic, that it seems more important than ever to obtain a more definite knowledge of them. The accounts of the strains reported as probably having an etiologic relationship to influenza are so meager that we cannot agree with a recent statement that these strains are "probably all the same organism," especially in the light of our work on colony fishings, the first report of which appears in this paper.

The most minute recent work on cultural characteristics of "green producing streptococci" has been done by Brown, confirming and continuing the work started by Theobald Smith and Brown in 1915. The chief advance that Brown made (1919) in studying more minutely the changes on a standard blood agar plate was to describe certain definite ice-box changes in the colonies. In his very comprehensive monograph he gave an extensive comparative table of bibliography and tried to correlate his work with that of others. He adopts, with some modifications, the classification of Holman as to the sugars.

His work on colony changes on his standard blood agar plate is most interesting in that he claims that certain small but definite appearances differentiate clearly between the so-called true hemolyzers (Smith and Brown's beta type) and the green-producing streptococci (Smith and Brown's alpha type).

We are using the term "alpha" as stated by Smith and Brown in 1915, although Brown in the introduction of his 1919 monograph says that they avoided the term *Streptococcus mitior seu viridans* for the less hemolytic streptococci because many of them produced little or no green color in blood agar plates. All of our strains reported in this paper were fished as green-producing colonies from Brown's standard-blood-agar plates. We are also accepting tentatively the broader description of hemolysis that Smith and Brown recognize, namely, that of a passing of hemoglobin from the red blood cells from whatever cause and that any lightening of the medium about a colony in a standard blood agar plate is evidence of a corresponding degree of hemolysis in this general sense.

As we said, the strains reported in this paper were originally fished from colonies that appeared to be of the alpha type and in the influenza cases all apparently of one type of alpha which we labeled "minute greens." These fishings were subsequently studied with great detail on blood agar plates following exactly Brown's method as reported in his monograph.

They were also studied for morphologic and staining characteristics after growth on chocolate agar and in pneumococcus broth. They were all Gram-positive cocci growing chiefly in medium-length chains in the broth and in pairs on chocolate agar. On the latter medium the individual organisms were frequently elongated. The morphology of the organisms in a few of the isolations differed in some particulars from that of others but whether these are lasting differences we do not know. The bile test in all of them was negative. Their reactions were tested to seven carbohydrates, namely, dextrose, saccharose, mannite, lactose, salicin, raffinose and inulin. Duplicate tests were made with the addition of serums from defibrinated horse blood.

Smith and Brown had described a sub-group of their alpha type based on colony formation which they called "complex." Brown described a sub-group based on blood change which he calls "alpha prime."

In general, we may say, our description of the types agrees with that of Brown. The slight differences we have found may be listed as follows:

1. All the strains we have studied as "alpha" produce a green zone, however delicate, on standard plates.
2. Some of our alpha strains have shown no increase in the zone of clearing after refrigeration.
3. Our alpha strains growing as "complex colonies" are rather frequent.
4. All of the strains, which we have classed as "alpha prime," have produced at least some green color within forty-eight hours in the incubator (36°C.).
5. Some of our alpha primes have produced some hemolysis in the test tube (reported to us by Mishulow).
6. An occasional definite "beta" strain has produced a green zone on the standard plate.
7. Two strains of "betas" have markedly complex colonies. (These are the strains marked "Witt"[2] and "Witt-like" in the report to be given by Valentine and Mishulow.)

We have come to the conclusion that the chief distinguishing characteristic between the alpha and beta type of hemolysis on Brown's standard blood agar plate is the zone of corpuscles immediately surrounding the colony, and that this characteristic may be a more constant test of the different types than tube hemolysis.

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[2] Two interesting organisms were obtained from the "Witt" case, with which different groups in our laboratory have worked. One is a hemoglobophilic bacillus (see Dr. Povitzky's paper) and the other is a hemolytic streptococcus.

We found that there was no consistent relation between types on standard blood agar plates and sugar reactions. Heterogeneity of sugar types found in colony fishings of "green streptococci" from individual cases of "common colds" has been reported by Krumwiede and Valentine.

The only general statement we can make so far from this study is that in a series of influenza cases, cultures isolated from colonies of an apparently similar type belonging to the "alpha streptococci and appearing as dominants, show a multiplicity of strains as indicated by the reactions with carbohydrates and with a standard blood-agar medium.

Although these cultural reactions may not be as fixed as certain immunological reactions, it remains to be demonstrated that these cultural reactions can change in their passage from individual to individual throughout a single epidemic.

For bibliography see the original article.

ON THE EXISTENCE OF A MULTIPLICITY OF RACES OF B. INFLUENZAE AS DETERMINED BY AGGLUTINATION AND AGGLUTININ ABSORPTION.

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Eugenia Valentine and Georgia M. Cooper

In the general investigation of the bacteriology of the recent epidemic of influenza, carried out under the supervision of Dr. Wm. H. Park and Dr. Anna W. Williams, it was early seen that one of the most important questions was the identity or non-identity of the strains of *B. influenzae* from the epidemic cases. Although the practically uniform presence of *B. influenzae* in actual cases of the disease suggested the etiological importance of this organism, no conclusion was possible without the further demonstration that the strains encountered were identical. It would be difficult to conceive a pandemic spreading from country to country, due to different races of a microörganism.

Preliminary observations indicated that the relationship of the strains isolated could be determined most easily by agglutination and agglutinin absorption. It was found that agglutinating sera, active in dilutions of 1—800 to 1—1000, could be produced without great difficulty by intravenous injection of rabbits, starting with live or killed bacilli. The technic employed is given in the original article.

The first strains selected for study, with one exception, were isolated at post mortem from lung or larynx. If there were an epidemic strain it seems that the strains having the ability to extend from the nasopharynx and invade the lung would most likely be the actual epidemic variety rather than an accidentally present parasitic type. As has been shown, the influenza bacillus is very frequently encountered in the nasopharynx of apparently normal individuals as well as in the throats of persons suffering from the diseases due to other agents. The preliminary strains were nine in number, and an agglutinating serum was prepared for each. For comparison, a culture of *B. influenzae* (B. I.) isolated in 1914 from the lung at autopsy in a case of pneumonia and a serum prepared against the culture were included.

The general absence of cross agglutination, or the slight degree when present, as shown in this series, indicates that even among these ten strains there exist dissimilar varieties.

To exclude the possibility that the cross agglutination obtained was any indication of close relationship or identity, agglutinin absorption was resorted to.

The results indicated that the ten strains studied were different one from the other, or in other words from nine cases of influenza, nine distinct races of the influenza bacilli were isolated. This result was so surprising that it seemed advisable to extend the study to another series of cultures, especially those isolated in the early stages of the disease, and to strains isolated from cases during convalescence, as well as those isolated from apparently normal throats. An additional series of cultures obtained post mortem from lung, larynx, or trachea, together with six strains isolated by means of mouse passage from sputa of suspected influenza pneumonia were included.

Two antisera prepared with nasopharyngeal strains isolated early in the disease were employed in addition to the nine sera employed in the previous series.

The results again indicated relatively little relationship between the strains employed and the strains used for the antiserum. In some instances, a considerable degree of cross agglutination was noted. To determine whether this indicated a close relationship or identity, absorption was resorted to.

In only two instances, was identity in absorptive capacity encountered.

Although these two instances of identity were encountered, the general absence of relationship of the strains from this series of cases to the strains used to produce the antisera was strongly suggestive of multiplicity. A possible explanation would be that not one of the serum strains used was an actual primary infecting epidemic strain but rather a secondary infecting strain of the same species. If *B. influenzae* were the actual primary etiological agent of the disease, the explanation just referred to would seem somewhat remote, in view of the origin of most of the strains from post-mortem cases which is evidence that the strains possess invasive power. To obtain more direct evidence as to the multiplicity of the strains of *B. influenzae* as well as on the question of the possible occurrence of primary and secondary infecting strains of this microörganism, two further series of cases were investigated.

Two series were studied, one from a group of marines who had lived for some time in an isolated camp, the other from the inmates of an infant asylum. Two or more isolations were made from a considerable number of the cases. If a definite strain was the primary etiological factor, it would not seem possible to fail to demonstrate a considerable degree of identity in the strains, in such series. Antisera were prepared with four cultures from the marine group and with one culture from the asylum group.

The cross reactions were no more marked than in the previous series with the exception of one antiserum of the marine group. This serum gave many marked cross reactions, several equalling or exceeding the reactions obtained with the homologous strain.

Agglutinin absorptions were again carried out in the instances where possible relationships were indicated. In the marine group, two strains from different individuals were found to have the same agglutinin absorptive capacity (M. 1 and M. 4). Two strains from different individuals were also found to be identical in the asylum series.

In spite of the marked cross agglutination shown by one antiserum, none of the strains from other individuals absorbed the specific agglutinins of this serum. In other words in spite of close contact no greater proportion of identical strains was encountered in this series than in the general series already described. A noteworthy finding is the result obtained with three successively isolated strains from a marine. Two earlier isolations were alike, whereas the later isolation was a different type. The appearance of different strains in the throat is not necessarily an indication of primary and secondary infection by two strains of the same bacterium. The individual may have possessed the two strains originally, or the second strain may have been implanted by contact.

An opportunity arose to study the strains isolated from the members of a single family all ill with influenza. The onset of the disease in the different members was very close together.

Antisera were prepared with the strain isolated from each individual. No identities were encountered. It would seem that in such a series infected from a common source or one from the other, identities should be encountered, if B. *influenzae* were the primary infectious agent.

TABLE 1

Summary of Investigation

SERIES	NUMBER OF STRAINS TESTED	NUMBER OF ANTISERA FOR GROUP	NUMBER OF OTHER ANTISERA	FINDINGS
Autopsy	10	10	7	All strains found to be distinct races. One autopsy strain identical with a miscellaneous strain. Another autopsy strain identical with another miscellaneous strain.
Miscellaneous	73	0	18	No two strains in this group were found to be identical. For identities see Autopsy Series above
Marines	54	4	14	Two strains from different individuals were found identical. Of isolations from the same individual: 4th day identical with 6th day 4th day identical with 8th day identical with 11th day 2nd day identical with 8th day 4th day identical with 8th day identical with 15th day 3rd day identical with 7th day, but 14th day was different
Hebrew orphans	28	5*	16	Two strains were found identical.
Family	6	6	10	All strains were found to be distinct races.

* The results with one serum are given in another table (see original article). The strains were tested with four other sera, but no additional identities were discovered.

DISCUSSION

The results recorded indicate that, under the term *B. influenzae,* we are dealing with a group of organisms which, for practical purposes, is heterogeneous in character as determined by immunological reactions. We have evidences of the existence of small sub-groups. The actual frequency with which identical strains would be encountered could only be determined by the use of many more sera than we have employed. Our own results, however, indicate at least that identical strains would be infrequently encountered.

The existence of a multiplicity of races is advanced as evidence that *B. influenzae* is not the primary etiological agent in epidemic influenza. To controvert this evidence, it would be necessary to prove either that the agglutinin absorption capacity of *B. influenzae* was a characteristic susceptible to rapid change or that, in no instance, have we utilized an actual primary infecting strain for the production of our serums. By analogy with the constancy of the agglutinin absorptive capacity shown by other varieties of bacteria, the former assumption is extremely improbable. The latter assumption that we have failed to employ an actual epidemic strain is rendered very remote especially by the employment of strains isolated early in the disease from groups of cases in close contact.

FURTHER STUDIES ON GROUPING OF INFLUENZA BACILLI WITH SPECIAL REFERENCE TO PERMANENCE OF TYPE IN THE CARRIER

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The first large series of experiments on the relationship of the influenza bacillus to epidemic influenza was carried out at the Research Laboratory under the direction of Park, Williams and Krumwiede. The serological work on grouping was done by Valentine and Cooper. They worked with 171 strains isolated by Williams from different sources during the pandemic of 1918. These strains were tested by the agglutinin absorption method with 25 antisera. The only grouping of strains into types, that could be obtained by this method was as follows: Strains from two individuals among a group of marines infected with influenza were found to be identical. Strains from two children at a children's home also gave identical reactions. As no other definite relationships could be established among the large number of strains tested, the conclusion seemed justified that under the term of *Bacillus influenzae,* just as with the term pneumococcus, we are dealing with a group of heterogeneous organisms. In the case of the influenza bacillus, there is even less suggestion of any dominant types. The existence of a multiplicity of types in cases attacked at the same time and in the same locality, was advanced as evidence against *B. influenzae* being the primary etiological agent in epidemic influenza.

Huntoon and Hannum state that there were no strains among their cultures which did not show relationship, either directly or indirectly through absorption tests. The titer of their serums was rather low for identifying strains by absorption tests. It is extremely probable that with monovalent serums of higher titer many more differences would have been discovered. Moreover, some of their tables show distinct type differences by absorption tests.

Small and Dickson studied ten strains and obtained four type groups from nine strains by subjecting them to agglutinin absorption tests.

Rivers from his investigations thinks that the influenza bacillus can be divided into groups according to their biological characteristics. Thus, he found one definite group of ten strains all of which were similar culturally, all formed indol, all reduced nitrates to nitrites and made blood broth milk slightly acid within forty-eight hours. Besides these he found some strains which produced hemolysis.

In spite of the general agreement in the results of the different investigations, it was thought wise to obtain a large number of new strains and separate them into their immunological types by direct agglutination and agglutinin absorption tests. We utilized about 100 old strains most of which had been isolated by Williams during the pandemic of 1918 and 90 new strains. These last had been isolated by one of us from a series of cases studied by Williams in connection with the recent outbreak of influenza. We had 30 sera, some of which were prepared from the strains of the pandemic, but most from the strains isolated previous to and during the recent outbreak. We tested all our old and new strains with these 30 sera at different times and we state at once that our results are in close agreement with those obtained by Valentine and Cooper. We added three more strains to the Leuchner group of Valentine and Cooper, thus identifying five strains in the Leuchner group (tables 1 and 2 of the original article). A great many other strains were agglutinated to a high titer by heterologous serums but by absorption tests were proven to be alike only in their abundant group agglutinins (table 3 of the original article).

As to the cultures isolated previous to and during the recent epidemic, none except a few contact cases were found to be identical in type, though a great many strains seemed to be closely related as shown by the absorption of a certain amount of agglutinins. We shall, however, speak about these strains from the recent outbreak in more detail later.

In contrast to the cultures from the respiratory tract we found a dominant type of B. influenzae from cases of influenza meningitis. In all, we had seven influenza meningitis cultures, all of which, except one, were isolated in our laboratory (Meningitis Division); of these, two had been isolated a few years before the pandemic, three during the pandemic and one since. One was given to us by Wollstein of The Rockefeller Institute. We developed anti-serums for two of the influenzal meningitis cultures, namely 6741 and 6746. Four of the seven influenzal meningitis cultures were agglutinated by the first serum (6741) and absorbed the agglutinins. None of the remaining three was agglutinated by or absorbed the second serum except the homologous strain. Culture 6746 is therefore, a distinct type. The remaining two cultures were not identical with either of the above types.

Before coming to the discussion of the recent epidemic, we must say a word about the difficulties encountered when working on agglutination tests with *Bacillus influenzae*. The difficulty Wollstein emphasizes, namely, that this organism clumps spontaneously, was with our methods negligible. Ever since the workers in this laboratory began to grow this organism on the chocolate medium and to scrape the growth off in salt solution, instead of washing it off, a homogeneous suspension is the rule. The greatest difficulty in the past has been the capriciousness and unreliability of the influenza bacillus as to its agglutinability. Some time ago, we noticed that the cultures used for animal inoculation and therefore transplanted each day, could be relied upon more surely for their agglutinability than the same cultures kept in stock and transplanted only once a weeek. In order, therefore, to restore to cultures their lost agglutinability we have transplanted them every day for at least a week before using them in a test. In the case of convalescent carriers, we noticed also that when we had a serum ready, the cultures if freshly passed through animals responded much more promptly to agglutination than they had done before or did a little later on. The loss in agglutinability was noticeable within a week's time after isolation. One of our cultures, namely Witt (isolated by Williams from a case of pleurisy following influenza and used by Cecil for his experiments) when tested, failed altogether to agglutinate, but the same culture when passed through the monkey agglutinated beautifully if transplanted every day for one week before doing the tests. As to the serum, if it is of good titer and agglutinates the culture in large clumps, it keeps for long periods without appreciable deterioration in the ice box. We have used such a serum over a period of two years without noting any considerable diminution of its titer.

Our technique is given in the original paper.

Since this paper was published 45 more cultures have been isolated from cases of Influenza Meningitis and we found that the same proportion still holds good, *i. e.*, fifty per cent. of these cultures fall into the dominant type group.

INVESTIGATION OF THE STRAINS FROM INFLUENZA-LIKE CASES IN RETURN OUTBREAK OF THE WINTER, 1920

The object of our studies was twofold: First, to test again the possibility of the constant presence of a dominant type of influenza bacillus which we had failed to find during the pandemic of 1918. If such a type were found, it might be considered the cause of the original pandemic as well as the return outbreak.

Second, to determine the persistence of type characteristics in the strains found in convalescent cases and in normal carriers of the influenza bacillus. If type characteristics were stable, then the finding of many different types in the epidemic cases occurring at about the same time would militate still more strongly against the etiological significance of the influenza bacillus.

With these objects in view, we fished from each case at least 10 colonies and from the selected cases as many as 30 of both small and large influenza-like colonies. Each colony from an Avery medium plate was transplanted on chocolate medium (reaction pH 7.2 to 7.4). Each culture from each separate colony was tested and retested as to its hemoglobinophilic properties by trying it on plain and ascitic agar and studied for its morphology. We utilized the morphology of the hemoglobinophilic organisms to separate the atypical from the typical hemoglobinophilic bacilli.

Atypical hemoglobinophilic bacilli. These are bizarre, long and curved bacilli, often growing out in threads. They seem to correspond to the organisms which Pritchett and Stillman name "the undescribed bacillus X." We found these organisms as the sole hemoglobinophilic organism in 18 cases (some normals and some colds) previous to and during the recent epidemic and in 5 cases coexistent with typical influenza bacilli. Therefore, we felt it worth while to study them more in detail. We found that on the chocolate medium they produced a mucoid growth. They all produced hemolysis, though in varying degree, in tubes with rabbits' washed red cells and in plates with defibrinated horse blood. The hemolytic colonies on superficial examination might be mistaken for hemolytic streptococcus colonies. These organisms do not appreciably ferment glucose as the majority give no reaction and a few show but a trace. They do not produce indol. By means of two sera produced by immunizing rabbits with two different strains, we were able by absorption tests to separate two groups of two and three each; the rest do not belong to either of these two groups and may or may not form many independent groups. Direct agglutination tests are not satisfactory as most of the bacilli clump spontaneously.

Typical hemoglobinophilic bacilli. The morphology of these was such as we usually associate with a typical influenza bacillus; a small slender rod often coccoid in form, later become more irregular and often growing out in threads. It grows profusely on the chocolate medium, between pH 7.2 to 7.4 or 0.2 to 0.3 acid to phenolphthalein (neutral to phenolphathalein before autoclaving).

In order to study the agglutinating characteristics of our separate colonies, we produced over thirty different sera from selected cases.

Rabbits chosen for inoculation weighed between 2000 and 2500 grams. Inoculations were given on three successive days if the condition of the animal allowed and three days rest followed. This process was repeated as often as necessary. An emulsion in saline of 1:40 to 1:10 of the growth on one test tube slant of chocolate agar was used for the initial injection and the amount gradually increased to the growth on two slants. The rabbits on the same culture were found to differ greatly in their resistance and power to form agglutinins. The average time necessary to obtain a serum giving complete agglutination in 1.2500 dilution was seven weeks and the average number of injections was 17.

TABLE 1

INFLUENZAL MENINGITIS GROUP OF CULTURES

DIRECT AGGLUTINATION								AGGLUTINATION AFTER ABSORPTION														
Serum	Culture	100	200	400	600	800	1200	Serum	Culture for absorption	100	200	400	600	800	1200	Serum strain	50	100	200	400	600	1000
6741	6741	+	+	+	+	+	+	6741	6741	–	–	–	–	–	–	6741						
	B. I. W.	+	+	+	+	+	+		B. I. W.	–	–	–	–	–	–	6741	–	–	–	–	–	–
	Z.	+	+	+	+	+	+		Z.	–	–	–	–	–	–	6741	–	–				
	747	+	+	+	+	+	+		747	–	–	–	–	–	–	6741	–	–	–	–	–	–
	6746	±	–	–	–				6746	–	–	–	–	–	–	6741	+	+	+	+	+	+
	6745	±	–	–	–				6745	–	–	–	–	–	–	6741	+	+	+	+	+	+
	6827	±	–	–	–				6827	–	–	–	–	–	–	6741	+	+	+	+	+	+
6746	6746	+	+	+	+	+	+															
	6741	±	–	–	–	–	–															
	Z.	±	–	–	–	–	–															
	6745	±	–	–	–	–	–															
	6827	±	–	–	–	–	–															
	B. I. W.	±	–	–	–	–	–															

Among our most interesting studies of the recent epidemic were those made on a family of five, a mother and four children. They were taken ill with influenza at the same time and were all brought to the hospital. From 10 to 20 colonies were fished from plate cultures of each member of the family. The interesting fact was brought out that the colonies obtained from each member of the family were not all identical with each other; the individuals had probably been carriers of two or more distinct types before the new infection. Homologous serums were prepared with organisms from two members of this family, starting with one colony from an individual in each case. These sera were tested with a number of strains from each case.

To sum up the family table (table 4 of the original article): all except two of the colonies fished from the mother's plates were identical with the serum strain. One of Alfred's colonies was identical with mother's majority strain; the other three were not. One of Theodore's and one of Margaret's colonies were identical with mother's majority strain. All the other strains were different. Four of Alfred's colonies were identical with Alfred's serum strain; three belonged to the atypical hemoglobinophilic bacilli producing hemolysis. One colony was identical with mother's majority strain; two colonies did not belong to any of the groups. One of Alfred's colonies and one of Margaret's colonies, tested by absorption, were identical with Alfred's serum strain.

Outside of this family, in other cases for which we had sera, many colonies were fished and usually showed the same type of organisms. Thus we fished from case 126, 30 colonies, large and small. In case 76, cultures were taken twice a week with a week's interval between; 10 colonies were fished each time. So also three sets of cultures were taken at week intervals from case 39; again 10 colonies were fished each each time. All colonies in each case proved to be identical by direct agglutination and agglutinin absorption with their homologous serums. The same was true of cases 101 and 132.

PERMANENCE OF AGGLUTINATION CHARACTERISTICS DURING DISEASE AND THROUGHOUT CONVALESCENCE

Carriers. Since the pandemic of 1918, Pritchett and Stillman, also Winchell and Stillman found influenza bacilli in 45 per cent. of the throats of convalescents and normal individuals. The duration of the carrier state lasted at times from four to five months. Opie, Friedman and Blake found *Bacillus influenzae* in over 35 per cent. of healthy throats in Camp Funston. They had no way, however, of telling whether the organism remained of the same type or was replaced by another type. The same question is brought up by Bloomfield. In 1918, immediately after the epidemic, we examined convalescent carriers with homologous serums which had been prepared at the time of infection. In one of these cases, still a carrier at the end of the second month, the type remained the same.

From a second case, 50 colonies were fished, 49 of which proved to be identical with the serum strain. One colony was not identical. Two to three weeks later, 10 more colonies were fished from the same throat and none of these were identical with the former 49 colonies, but all were identical with the one strain which was different from the 49. In one case at the Hebrew Orphan Asylum, a culture taken one year later has proved by agglutination and agglutinin absorption tests to be of the same type as that of the year before. But in another case at the same asylum, the culture was of a type different from that of the year before.

In a few of our new cases just before the winter outbreak (for example, case 39 in which cultures were taken once a week for three weeks) the type remained the same by serological tests. The same was true of case 76; two subsequent fishings, one week apart proved to be the same, as well as the organism recovered from the mouse. The cases of accidental infection, reported in paper 5 of these studies, are of importance not only because due to cultures but also because of the proof of the stability of type during the infection.

A most interesting illustration of this is a case of Cecil in his experiments on the infectivity of *Bacillus influenzae.*. A volunteer, Loselle was infected with the exudate from the monkey which had been inoculated with one of our pleurisy cultures (Witt). For almost three months he remained a carrier of that same type of organism (see table 5 of the original article.). On the other hand, another of Cecil's cases, Burns, who was a carrier before he was inoculated with culture Witt which had been recovered from the above case, showed on the third day the following among seven colonies: two colonies composed of strains identical with Witt and one unlike Witt; also four colonies which were mixtures of Witt and other types. One week later, no colonies of the Witt type were found. Another of Cecil's cases, Small, inoculated with the Witt strain, showed the culture to be still of the same type after the fifth day.

Crane (Cecil case) was tested culturally at intervals after inoculation with the Witt strain. This strain was found to be present at the end of one hour and also on the third day; but one week later, after a relapse, the culture taken was not of the Witt type when tested by direct agglutination and by agglutinin absorption. The later disease, if due at all to influenza bacilli, was probably due to a new infection.

Cultural types by indol tests. Jordan found that 10 out of 13 strains of *Bacillus influenzae* formed indol. We were able to corroborate his tests by using the paradimethylamido benzaldehyde method that he employed. Out of 42 typical influenza strains, 28 gave indol and 2 more gave traces of indol. None of the atypical hemoglobinophilic bacilli formed indol. We selected our cultures from among different groups in order to see how far the serological tests would correspond with the indol tests and glucose fermentation tests (see table 6 of the original article: meningitis group, Leuchner group, family group).

In the Leuchner group no cultures formed indol. In the meningitis group, three formed indol and one did not although they all were identical serologically; in the family table, all the cultures tested showed indol except those which belonged to the atypical hemoglobinophilic bacilli.

SUMMARY

1. Four out of seven influenzal meningitis strains isolated years apart, were proved by agglutinin absorption tests to be of one type; the remaining three were of different types. From the respiratory cases, five strains from different individuals were obtained which were of the same type. No other groups of more than two members were obtained in agglutination tests with any cultures. This was true of both the original pandemic and also of the new outbreak strains.
2. Atypical hemoglobinophilic bacilli were found in 18 cases (normal individuals and cases of cold) and in five cases these were found in coexistence with typical influenza bacilli. These atypical organisms produced hemolysis, did not form indol and did not ferment glucose.
3. About 75 per cent of typical influenza bacilli form indol; from 50 to 60 per cent. ferment glucose somewhat irregularly and not energetically.
4. The indol reactions did not correspond fully among the meningitis group of four serologically identical strains. Of these, three formed indol—one did not. In the family group, though the strains were not all identical serologically, all except the atypical hemoglobinophilic colonies formed indol. In general, it was found that strains having the same immunological characteristics had the same cultural reactions, but there were some exceptions.
5. Many colonies were fished from each of a number of selected cases and tested with homologous and heterologous serums. In a few cases as many as 20 or 30 colonies from each case were tested with the homologous serum prepared from one of these colonies. In most cases the fishings of influenza bacilli proved to be of one type; at least one type greatly predominated. But in a minority of instances, the colonies from the same case were not in the same type as proved by agglutination absorption tests.
6. In carriers or where the infection was mild, more than one type variety of the influenza bacillus were apt to be found in the same case.
7. In several convalescent carriers, tests were made during periods of from several weeks to three months. These resulted in finding in all but one, the original type of the influenza bacillus. In one case, tests made one year apart proved the original type present.

For bibliography see the original article.

ANNOTATION: The study of influenza bacilli isolated from cases of meningitis has been continued.

As mentioned above, four strains out of seven fell into one group by agglutination and absorption tests. For this work we used a monovalent serum from rabbits immunized against the oldest strain of that group; namely, Bacillus influenzae w. Since that time we have obtained, from Dr. Neal, influenza cultures isolated from cases of meningitis as follows:

1922	13 cultures
1923	6 cultures
1924	5 cultures
1925	3 cultures
1926	6 cultures
1927	2 cultures
Total	35 cultures

About 50% of these cultures continue to fall into the same group which is now designated as the "Predominating Influenza Meningitis" group or Type I. Two horses have been immunized with the strains of this predominating group and the serum used therapeutically in cases of influenzal meningitis.

ACCIDENTAL INOCULATION OF INFLUENZA BACILLI ON THE MUCOUS MEMBRANES OF HEALTHY PERSONS WITH DEVELOPMENT OF INFECTION IN AT LEAST ONE. PERSISTENCE OF TYPE CHARACTERISTICS OF THE BACILLI.

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The three accidental inoculations of influenza bacilli (eighteen hour cultures on chocolate agar), are of interest from the fact that infection developed in one case on the fourth day; possibly in one case on the fourteenth day and not at all in the third case. All three persons, however, became persistent carriers of the bacilli and in all cases the strains held their agglutination characteristics unchanged during the period of the tests. The details of three accidental inoculations are as follows:

Case I. It is difficult to judge whether this case was really one of infection from inoculation with the Pfeiffer bacillus or not because of the long period of incubation. Dr. H. accidentally inoculated her throat with the Lee culture, an autopsy strain from an epidemic case, while expelling a culture suspension from a syringe. The culture was an eighteen-hour growth on "chocolate blood" mixed with human serum. It happens that some time before the accident a culture from Dr. H. had been made and gave no influenza bacilli. No symptoms developed for two weeks when she developed a temperature of 101, prostration and general pains. She was confined to bed. On her return, plate cultures made from the nasopharynx showed abundant influenza bacilli.

Ten colonies were fished from a pharyngeal culture.

All isolations were alike and identical in type with the accident strain. The direct agglutinations are given in table 1. Two of the strains were

TABLE 1

AGGLUTINATION OF H STRAINS (EXAMINATION I) BY LEE SERUM

STRAINS	SERUM DILUTIONS						CONTROL
	1:50	1:100	1:200	1:400	1:700	1:1000	
H-I-1	+	+	+	±	×	−	−
H-I-2	+	+	+	±	×	−	−
H-I-3	+	+	+	±	×	−	−
H-I-4	+	+	+	±	×	−	−
H-I-5	+	+	+	±	×	−	−
H-I-6	+	+	+	±	×	−	−
H-I-7	+	+	+	±	×	−	−
H-I-8	+	+	+	±	×	−	−
H-I-9	+	+	+	±	×	−	−
H-I-10	±	+	+	±	×	−	−
Lee	+	+	+	±	×	−	−

+ = Complete agglutination.
± = Marked agglutination.
× = All reactions weaker than those above.
− = No reaction.

further investigated by agglutinin absorption tests with the Lee serum (table 2). The identity of the Lee cultures and the H. strains was confirmed. Sixty-eight days after the accident 11 more strains were isolated from a nasopharyngeal culture. These were found identical with the previous autopsy and H. strains (table 3).

TABLE 2

AGGLUTININ ABSORPTION TESTS WITH H STRAINS (EXAMINATION I) AND LEE SERUM

STRAINS	AGGLUTINATION BEFORE ABSORPTION						ABSORBED BY STRAINS	AGGLUTINATION AFTER ABSORPTION												CONTROL
								Absorbing strain						Serum strain						
	1:50	1:100	1:200	1:400	1:700	1:1000		1:50	1:100	1:200	1:400	1:700	1:1000	1:50	1:100	1:200	1:400	1:700	1:1000	
H-I-3	+	+	+	±	×	—	H-I-3	—	—	—	—	—	—	—	—	—	—	—	—	—
H-I-7	+	+	+	±	×	—	H-I-7	—	—	—	—	—	—	—	—	—	—	—	—	—
Lee	+	+	+	±	×	—	Lee	—	—	—	—	—	—	—	—	—	—	—	—	—

Case II. Miss B. accidentally sucked into her mouth some salt solution suspension of an eighteen-hour culture on blood agar. She developed symptoms of a pharyngeal infection on the fourth day. There was a slight rise of temperature and moderate prostration. On her return to work nasopharyngeal cultures yielded influenza bacilli identical in type with those in

TABLE 3

AGGLUTINATION OF H STRAINS (EXAMINATION II) BY LEE SERUM

STRAINS	SERUM DILUTIONS						CONTROL
	1:50	1:100	1:200	1:400	1:700	1:1000	
H-II-1	+	+	+	±	×	—	—
H-II-2	+	+	±	±	×	—	—
H-II-3	+	+	+	±	×	—	—
H-II-4	+	+	+	±	×	—	—
H-II-5	+	+	+	±	×	—	—
H-II-6	+	+	+	±	×	—	—
H-II-7	+	+	+	±	×	—	—
H-II-8	+	+	+	±	×	—	—
H-II-9	+	+	+	±	×	—	—
H-II-10	+	+	+	±	×	—	—
H-II-11	+	+	+	±	×	—	—
Lee	+	+	+	±	×	—	—

the Holmes culture (table 4). Holmes was one of a group of marines among whom influenza developed some weeks before.

TABLE 4

AGGLUTININ ABSORPTION TEST WITH B STRAIN AND HOLMES SERUM

STRAINS	AGGLUTINATION BEFORE ABSORPTION						ABSORBED BY STRAINS	AGGLUTINATION AFTER ABSORPTION												CONTROL
								Absorbing strain						Serum strain						
	1:100	1:200	1:400	1:600	1:800	1:1000		1:100	1:200	1:400	1:600	1:800	1:1000	1:100	1:200	1:400	1:600	1:800	1:1000	
B	+	+	+	+	+	+	B	−	−	−	−	−	−	−	−	−	−	−	−	−
Holmes	+	+	+	+	+	+	Holmes	−	−	−	−	−	−	−	−	−	−	−	−	−

Case III. Miss R. was accidentally infected with Marine 3 culture, a fourth day case strain isolated from the nasopharynx. She received infection in the same manner as Dr. H. Miss R. was not ill after the accident. Twelve strains isolated from plate cultures made twenty-eight days after the accident were all alike and were identical with the accident strain (tables 5 and 6).

Case IV. Miss C was accidentally exposed in a manner similar to Miss R. and Dr. H. but she neither developed symptoms nor became a carrier.

TABLE 5

AGGLUTINATION OF R STRAINS BY MARINE 3 SERUM

STRAINS	SERUM DILUTIONS						CONTROL
	1:50	1·100	1:200	1:400	1:700	1:1000	
R-1	+	+	+	+	+	±	−
R-2	+	+	+	+	+	±	−
R-3	+	+	+	+	+	±	−
R-4	+	+	+	+	+	±	−
R-5	+	+	+	+	+	±	−
R-6	+	+	+	+	+	±	−
R-7	+	+	+	+	+	±	−
R-8	+	+	+	+	±	±	−
R-9	+	+	+	+	+	±	−
R-10	+	+	+	+	+	±	−
R-11	+	+	+	+	+	±	−
R-12	+	+	+	+	+	±	−
Marine 3	+	+	+	+	+	±	−

TABLE 6

AGGLUTININ ABSORPTION TESTS WITH R STRAINS AND MARINE 3 SERUM

STRAINS	AGGLUTINATION BEFORE ABSORPTION						ABSORBED BY STRAINS	AGGLUTINATION AFTER ABSORPTION												CONTROL
								Absorbing strain						Serum strain						
	1:50	1:100	1:200	1:400	1:700	1:1000		1:50	1:100	1:200	1:400	1:700	1:1000	1:50	1:100	1:200	1:400	1:700	1:1000	
R-3	+	+	+	+	+	±	R-3	−	−	−	−	−	−	−	−	−	−	−	−	−
R-11	+	+	+	+	+	±	R-11	−	−	−	−	−	−	−	−	−	−	−	−	−
Marine 3	+	+	+	+	+	±	Marine 3	−	−	−	−	−	−	−	−	−	−	−	−	−

CONCLUSIONS

1. Freshly isolated Pfeiffer bacilli are capable of causing infection when transferred in great numbers to susceptible mucous membranes.

2. Strains recovered from infected persons at varying intervals after the accidental inoculation, revealed agglutinative characteristics identical with those of the strains which had caused the infection. The varying periods cover the time from shortly after infection to convalescence. This is strong evidence that a strain responsible for an infection would reveal identical characteristics during infection and convalescence in the epidemic cases due to its invasion. If the epidemic of influenza were due to the Pfeiffer bacillus, we would expect the dominant strains to have similar type characteristics. The fact that just the opposite condition exists is strong proof against the primary etiological importance of the Pfeiffer bacillus in epidemic influenza.

REPORT ON THE PROPHYLACTIC VACCINATION OF 1536 PERSONS AGAINST ACUTE RESPIRATORY DISEASES, 1919-1920

Published in the Journal of Immunology, 1921, Vol. VI, pp. 103-115

Anna I. Von Sholly and William H. Park

The reason for the investigation upon which this report is based, was to attempt to clear up, if possible, the status of a vaccine made of the mixed organisms which predominate in respiratory diseases, used as a prophylactic agent in epidemic "grippe" and acute respiratory diseases in general. Reports on vaccine prophylaxis are only too plentiful both for vaccine made of pure *Bacillus influenzae* and for a vaccine made of various mixtures of the important microbes found in the respiratory tract. The chief criticism to the recognition of their claims is that they were tested out during the late pandemic of "grippe" either close to or after the peak of the epidemic, at a time when it was impossible to sort out cases into convincing statistical groups on account of the unknown factor of natural or inherited immunity which plays an important and as yet little charted part in epidemiology. Because, too, of this unknown denominator of natural immunity, adequate control groups, for scientific comparison with the inoculated groups, during the epidemic period, were not obtainable.

Our aim, then, in this study, was to inoculate prophylactically a group of people well in advance of any anticipated recurrence of epidemic grippe so that whatever immunity might be stimulated by this method would be already established before exposure to the disease. Moreover, we wished to learn what effect a vaccine made of the predominant organisms of the acute respiratory diseases would have in inhibiting or modifying these diseases. We aimed, too, to have for comparison an uninoculated control group as similar as possible to the group inoculated.

The work presented was done between September 30, 1919, and April 3, 1920, among the general employees of the Metropolitan Life Insurance Company's Office, New York City, under the direction of the Research Laboratory of the New York City Health Department, coöperating with the officers of the Metropolitan Life Insurance Company. This company gave the services of its nursing and clerical forces and defrayed the cost of the vaccine. The groups under observation consisted of men and women, all volunteers, of varying ages. In all, there were 1536 people who were inoculated, of whom 1293 received three inoculations, 95, two inoculations, and 148 only one inoculation. As a control group 3025 persons of similar ages and both sexes of the same company, were under continuous observation. Only about 13 per cent. of the original inoculated group and 14 per cent. of the uninoculated dropped out during the six months of investigation.

The personal history, home conditions and habits of the entire group as given in the original form filled out by each volunteer at the beginning of our work, summarized in percentages taken from 1500 histories selected at random shows similarity between the two subgroups; i.e., inoculated and uninoculated.

METHOD OF FOLLOWING THE GROUPS

On entering the group, each person filled out a form, which was kept under his or her name in a filing cabinet. All subsequent records of each person were attached to this form as they were collected. That we might not slip up in following the course of each one's health, we imposed a series of checks in the collection of the material. A form was given each person to fill out and return when he or she was suffering from any acute respiratory disease. Still another form was given section heads who were made responsible for their weekly return to us with the names and condition of those in the section suffering from "colds." In addition, copies were made daily from the dispensary records of all persons who had reported for treatment for any respiratory disease during that same day. For several months, many of these people were seen personally by one of us (von Sholly) in order to obtain material for culture work. Furthermore, from the Medical Welfare Bureau which has supervision over all employees who are absent for illness, we obtained daily reports, with diagnoses of the disease and length of absence. Pneumonia cases were investigated to confirm the diagnosis. All the facts obtained were inscribed daily on the records and filed away. To indicate the extent of the statistical work, we may state that we had one doctor, one nurse, two whole time clerks, two part time clerks for a fairly long period, and one laboratory assistant at work daily, for more than six months. We do not consider our records perfect. They could not be so, where one had to deal with a freely moving volunteer group of varying degrees of intelligence and coöperation. We hope, however, that they are better than many that have been offered. Undoubtedly we did not get a record of every "cold" experienced in the group but feel confident that whatever errors have crept in were true of both groups, inoculated and not inoculated, and that, with the check of the absentee and dispensary reports, only the mildest affections requiring slight treatment and no absence are not recorded. The individual diagnoses, too, may not be strictly accurate. Some were self diagnosed (checked as far as possible by the Company's physician). Furthermore, the diagnoses of outside physicians may not conform absolutely to our nomenclature. By grouping the infections, however, into upper and lower respiratory diseases, we think they are fairly accurate—sufficiently so for the purposes of this investigation.

The injections of vaccine were given at weekly intervals during the end of September, 1919, and during the first three weeks of October, 1919. Two vaccines[1] were used. The larger group of persons numbering 1412, were given vaccine *L,* a saline suspension sterilized by heat and made up of:

	million per cc.
B. influenzae	1000
Streptococcus hem	1000
Streptococcus vir	1000
Pneumococcus 1	2000
Pneumococcus 2	2000
Pneumococcus 3	1000

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[1] These formulae were selected by a committee composed of Dr. Park of the Department of Health, City of New York, Dr. Rosenau of Harvard, Dr. McCoy of the United States Public Health Service, and Professor Jordan of Chicago University.

The smaller group, numbering 124, were given a vaccine *R,* a vaccine similar in composition to that given by Rosenow of the Mayo Clinic. It consisted of:

	million per cc.
Pneumococci type 1 (10 per cent.), 2 (14 per cent.), 3 (6 per cent.)	1500
Pneumococci, group 4 and allied green producing Diplostreptococci	1500
Hemolytic streptococci	1000
Staphylococci (aureus)	500
B. influenzae	500

We kept a record of the reactions to the vaccines given, partly for general scientific knowledge and partly for the purpose of finding out the practicability of group vaccination for a commercial company from the point of view of loss of services following and due to inoculations. The injections were given subcutaneously in the deltoid region. The first dose was 0.5 cc. and the second and third were 1 cc. each.

There were, following the first injection, mild local reactions (area of redness not more than a silver dollar in size, and very slight soreness) among 63.8 per cent. of the group; moderate local reactions, (area of redness from size of a silver dollar to size of hand and slight soreness) in 11.8 per cent. of group; marked local reactions, (anything larger than moderate) in 3.3 per cent. of the group and no reaction at all in 21 per cent. Following the second injection (double the dose) mild local reactions were observed in 63.2 per cent. of the group, moderate in 21.4 per cent., marked in 2.7 per cent. and no reactions in 12.7 per cent. Following the third injection, mild local reactions were seen in 60.6 per cent., moderate in 9.7 per cent., marked in 4.1 per cent., and none in 25.5 per cent.

Of the general reactions, the mild ones, consisting of headaches, slight dizziness, nausea or general malaise were reported after the first injection, in 19.4 per cent. of the group; after the second injection in 27 per cent. and after the third in 23.7 per cent. The marked reactions, chills or chilly feelings, fever, general pains, or mild prostration, after the first injection, were observed in 3.1 per cent.; after the second, in 3.2 per cent. and after the third, among 2.6 per cent. Only 26.2 per cent. of the total inoculated gave general reactions. These figures show that the largest number of reactions, as was to be anticipated, followed the second injection and that the severe reactions were comparatively few. There were no really alarming reactions. The details of the reactions of the vaccines are given in table 1 in the original article.

That some people should take advantage of the inoculations to remain at home would not be at all surprising, especially, where there is an illness insurance. If we give everybody the benefit of the doubt and count all absences immediately following vaccination as due to it, there were in all 87 persons who were absent from work anywhere from a half day to 24 days. The total days absence of this group was 181½ days, or 2 days and 42 minutes for each one. The average absence per person for the total inoculated group, 1536 persons, taking the working day as 8 hours, was about 56 minutes. We may compare these percentages with the absences due to respiratory diseases during the six months observation period. The uninoculated lost 8172 days or an average of 2.2 days per person and the inoculated lost 2456 days or an average of 1.59 days per person during the same interval. If we add the average of the inoculated persons' absence due to respiratory disease to the average absence credited to inoculations, we still find a gain of a good half working day per person in favor of the inoculated. It is fair also to assume that if the custom of annual vaccinations were established, absences due to inoculations would diminish.

A tabulation was made to learn whether there was any demonstrable relationship between past respiratory diseases, such as pneumonia, bronchitis, grippe, etc., and the character and severity of the reactions from the inoculations. No especial susceptibility to vaccines can be established for these diseases. The same was true for the "allergic" diseases, asthma, hay fever, etc., but the numbers in our group are probably too small to show anything.

Respiratory Affections Among the Vaccinated and Unvaccinated During the Six Months Observation Period October 1, 1919, To April 3, 1920

There were 1327 completed records of inoculated persons under continuous observation during the above period and 3025 similar records of the uninoculated. Among the inoculated, 13.7 per cent. give a history of *no respiratory affections* during this time. Among the non-inoculated 29.77 per cent. give a similar history.

The respiratory infections which occurred in each group and their severity are tabulated in table 3 of the original article.

One might infer from the tabulated figures that it were wiser not to be inoculated. One must remember, however, that the observed groups were volunteers. Naturally, those volunteering for inoculation might be supposed to be the more susceptible to respiratory diseases, hoping for relief from the inoculations. And, in fact, our summaries of the original questionnaire show that 21.6 per cent of the non-inoculated were comparatively free from respiratory troubles in their past, as against 10.5 per cent of those who submitted to inoculations.

It is probably fair to conclude that, considering the results as a whole, the kind of vaccine use by us, in the manner and dosage indicated, had very little specific influence in preventing or causing respiratory diseases other than pneumonia.

Pneumonia

The difference of pneumonia incidence, however, is marked in the two groups.

The inoculated show 1 case (0.075 per cent) against 11 cases (0.36 per cent) among the non-inoculated, or about five times as many pneumonias among the non-inoculated.

It is true that the number of cases of pneumonia occurring in the group is so small that the apparently favorable results of vaccination may be due to chance, nevertheless, it would seem fair to make some claims for the efficacy of a typed pneumo-streptococcus vaccine as a preventive of pneumonia. The one case of pneumonia which occurred in the inoculated group. was a girl who had had only one injection of the vaccine on October 2, 1919. She suffered from influenza and broncho-pneumonia March 23, 1920, more than five months after vaccination. She was absent from her work just one month. She had had an acute bronchitis (absent 20 days) in January, 1920. Lister in South Africa does not claim much, immunity resulting from the pneumococcus vaccine for more than four to six months. After our investigation had been closed, two more cases of pneumonia, were reported among the inoculated. Both occurred in May, 1920, about seven months after the vaccinations. One of these patients had had two and the other three injections.

Of the 11 cases of pneumonia occurring among the non-inoculated, before April 3, 1920, seven were lobar in type and four were broncho-pneumonia. Two of the lobar pneumonias and two of the broncho-pneumonias were said to complicate grippe. One case of lobar pneumonia (primary) died after three days illness. In May 1920, a twelfth pneumonia was reported also among the non-inoculated. If we count these later cases, the percentage was still twice as high for pneumonia among the non-inoculated persons.

Grippe Cases

One of the most important phases of our investigation was the completion of the vaccinations about three months before the brief flare up of grippe in January and February, 1920. In the inoculated group, there were 232 grippe cases[2] during the six months, most of them occurring during January and February. These made up 17.8 *per cent.* of the inoculated group. Of these cases 49 (21.1 per cent.) had had grippe in the pandemic of the previous year (1918-1919), 191 of the 232 had been inoculated three times (in October, 1919), 14 had received only two injections and 26 only one injection.

Among the uninoculated, there were 494 cases of grippe, excluding the pneumonias, reported in the period of observation. These were 16.3 *per cent.* of the group. Eighty-eight persons (17.8 *per cent.*) had had influenza also during the previous year. In comparing these groups, we find very little difference in their respective degrees of immunity to what was considered as a recurrent outbreak of influenza.

Looking at these same figures from the point of view of the 1918-1919 epidemic forward to the 1920 return, the results are practically the same. Of the group reporting grippe in 1918-1919, 12.2 *per cent.* had a second attack in 1920 after inoculation, and 13.1 *per cent.* had a second attack, although not inoculated.

Most of the grippe cases of 1920 were comparatively mild. The inoculated averaged 7.2 days absence per person, and the non-inoculated averaged 9.2 days per person. The difference can be accounted for only to a slight degree by the fact that the pneumonias occurred more frequently among the non-inoculated.

Severity of Illness

It was a rather difficult matter to gauge accurately the severity of the various infections. One had to rely partly on the personal opinion of the patient. However, we made an attempt to judge by an arbitrary classification, as follows: Illnesses were considered severe that showed (1) fever over 102°, (2) confinement in bed for four days or more, (3) marked specific clinical symptoms, (4) some prostration. Moderate illnesses were those with (1) fever 100° to 102°, (2) confinement in bed or at home two to four days, (3) moderately severe specific clinical symptoms, (4) malaise. Mild infections were those with (1) a temperature less than 100°, (2) absence less than two days, (3) moderate or mild specific clinical symptoms, (4) slight or no malaise. Our statistics for severity of disease were taken from the absentee reports which, obviously, were the less difficult to judge, since all absentees are visited in their homes by the Company's nurse if they are away more than three days, and all are seen by a nurse or the Company's doctor on their return to work.

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[2] After these figures were obtained, there were a few doubtful cases subtracted —which however made practically no difference in the relative percentages.

In the inoculated group, 445 (30.8 per cent.) were absent during the period of October 30, 1919 to April 3, 1920. In the not inoculated group, 1235 (36.1 *per cent.*) were absent. If we divide these absentees into groups according to the degree of severity of their illness, we find 70.8 *per cent.* of both the inoculated and the uninoculated fall into the mild class; 26.7 *per cent.* of the inoculated against 25.7 *per cent.* of non-inoculated fall into the moderate class, and 2.4 *per cent.* of the inoculated against 3.4 *per cent.* of the uninoculated fall into the severe class.

From the examination of the foregoing stastistics, we may draw contrary conclusions depending upon whichever part of the report we lay more stress. If we compare the incidence of the respiratory affections among the inoculated and the uninoculated, excluding pneumonia, and if we consider the severity of these respiratory affections, we may assert that the vaccines were of little or no appreciable value. If, on the other hand, we focus our attention on the figures that show a somewhat higher susceptibility to respiratory diseases among the vaccinated in their past histories; a larger group absence for respiratory diseases among the not vaccinated and a longer average absence per person for attacks of "grippe" in the not inoculated group, we may conclude that the vaccines had a beneficial influence. On the whole, balancing both sides, our evidence does not make a strong case in favor of the vaccines given us as a prophylactic agent against acute respiratory diseases—pneumonia alone excepted. Our work tends to confirm the conclusions of Lister and Cecil and Austin on the value of a typed pneumococcus vaccine for pneumonia. It points to the importance of further research along this line.

TABLE 1

SEVERITY OF RESPIRATORY DISEASES AMONG ABSENTEES ON ACCOUNT OF SICKNESS

MILD AFFECTIONS	MODERATE AFFECTIONS	SEVERE AFFECTIONS	TOTAL PERSONS	PER CENT WHOLE GROUPS ABSENT FOR RESPIRATORY SICKNESS
Inoculated group				
315 (70.8%)	119 (26.7%)	11 (2.47%)	445 (100%)	30.8%
Not inoculated group				
874 (70.8%)	317 (25.7%)	42 (3.4%)	1235 (100%)	36.1%

Thanks are due for the help and courtesy extended to us by the staff of the medical and statistical departments of the Metropolitan Life Insurance company.

For bibliography see the original article.

STUDIES ON VACCINE THERAPY FOR PNEUMONIA AFTER 1920.

Anna I. Von Sholly

In April, 1921, the New York City Health Department, began to try out at one of the large State Hospitals the value of a combined Type I, II and III pneumococcic vaccine as a prophylactic. This trial was continued for a period of three years. The average daily census of the inmates of this institution was about 6,800 persons. One half were inoculated and the other half held as controls. The aged, feeble and tuberculous were excluded, so that a little less than 3000 persons were included in each group, the inoculated and the controls respectively. Several series of re-inoculations were given, some groups after six months intervals and other groups after one year intervals. The total dosage (divided into three injections at 5-7 day periods) was 30 billion in 1921-22, 22½ billion, in 1922-23 and 36 billion in 1923-24.

During the three years' study there were 12 cases of lobar pneumonia among the inoculated with 4 deaths and a similar number (12) among the unvaccinated controls with 8 deaths. Unfortunately however, on account of the difficulty of obtaining the co-operation of the patients, we were able to type the sputums of only three cases among the inoculated and only 5 among the controls. The three cases typed in the inoculated group (all of whom recovered) were two cases of Type III, (inoculated 7 weeks and 5 months respectively, previous to their illness), and one case of Type II inoculated 6 weeks before. None of the fatal cases in the inoculated group was typed.

The five typed cases among the control group were one case Type II, one case Type III and 3 cases Type IV. Three died, one of each of the above type cases.

The intervals that elapsed between the last series of inoculations and the attack of pneumonia among the inoculated who recovered were as follows:— three, within 5-7 weeks after the last series of inoculations, one, 3 months after, one 4 months after and three 5 months after. The four that died had received their last series, 3, 5, 6 and 7 months previously.

Terminal pneumonias and acute broncho-pneumonias are not included in the above report. According to Lister and Cecil and Austen, Type I, II and III pneumonias should not have occurred after inoculation. While our results are not as brilliant as those obtained by these investigators, they are not wholly discouraging. The incidence of pneumonia and the occurrence of the fixed type pneumonias were apparently not lessened by previous inoculations with the pneumococci, the mortality, however, seems to have been influenced very decided for the better.

STUDIES ON ACUTE RESPIRATORY INFECTIONS

A Study of the Cultural and Serological Relationship of Hemolytic Streptococci Isolated from Inflammatory Conditions Of the Respiratory Tract[1]

Published in the Journal of Immunology, 1921, Vol. VI, pp. 301-328

Eugenia Valentine and Lucy Mishulow

This study is part of the general investigation of the diseases of the respiratory tract. The purpose of this study was to determine whether or not a dominant variety of hemolytic streptococcus could be isolated from the cases of acute colds and influenza studied.

The rôle played by the hemolytic streptococcus in a considerable percentage of the fatal pneumonias in 1918 has stimulated many investigators to study the incidence of this organism in the secretions of the nose, throat and in the sputum of normal persons and in conditions of respiratory disease. They also studied the fermentative reactions in an attempt to correlate the fermentative types with the character of the respiratory condition.

The work of these investigators is of interest primarily in that it indicates that the streptococcus hemolyticus is frequently present in the throat, especially if the tonsils be large and cryptic. As to the correlation of definite fermentative types with the conditions in which they were found, the results are not altogether concordant. These results bear only indirectly on our problem, as the fermentative reactions give only broad primary groups, which groups are further divisible by immunological reactions.

Several investigators have studied the immunological reactions of the streptococci isolated from respiratory diseases. Most noteworthy is the work of Dochez, Avery and Lancefield who studied the immunological relationship of hemolytic streptococci especially from cases of pneumonia, many of which were secondary to measles. The material was obtained mainly from the military establishments about Fort Houston. These authors added to the evidence already available that fermentative differences are strongly indicative of immunological differences, whereas fermentative similarity is not necessarily parallel by immunological similarity. The earlier studies, especially those of septic sore throat (Smith and Brown, Krumwiede and Valentine) had indicated this.

In our attempt to find a dominant strain of streptococcus hemolyticus in this series of cases, the plan was to study first the general biological characteristics in order to determine what groups were separable by fermentative and hemolytic reactions. The members of these groups were then studied immunologically by agglutination, and where strains were seemingly alike, agglutinin absorption was employed as the final criterion of identity or non-identity.

The cultures studied were obtained from the nose, naso-pharynx or throat in acute colds, rhinitis and pharyngitis, and from the more severe influenzal conditions. A few pleurisy and mastoid cases are included, as well as normal controls. In many instances several examinations were made from the same case both during illness and after recovery.

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[1] One of a series of studies carried out under the direction of William H. Park, Anna W. Williams and Charles Krumwiede. The previous studies in this series were reported in the Journal of Immunology, vi, no. 1, January, 1921. This investigation was made possible by a grant of money from the Metropolitan Life Insurance influenza fund for a part of the expense.

The method of isolation of the organisms for this study was as follows:

A sterile cotton swab was rubbed over the surface of the mucous membrane of the nose, nasopharynx or tonsils and the material thus collected was rubbed up in broth which was used for "shake" pour blood plates. Some of this broth suspension was also inoculated intraperitoneally into white mice. If these succumbed, pour blood plates were made from the heart's blood. Material obtained by nasal irrigation with Ringer's solution was also used for pour blood plates. The blood plates were incubated for forty-eight hours and the colonies showing definite macroscopic zones of hemolysis were fished for further study. The strains were kept on blood-streaked agar and transferred fortnightly pending the comparative tests.

The data as to the individual case, the type of disease, the source of the culture, how obtained (from mouse or direct plate), and the number of examinations made on the patient are given in tables 1 to 6 of the original article. The scheme used for numbering the cultures is exemplified by the following complex numbers, 112, II, 4. The first number refers to the case from which material was obtained for bacteriological examinations. As hemolytic streptococci were frequently not obtained there is no sequence in these numbers. The second or Roman numeral refers to the different specimens taken at different intervals from the same patient. In the majority of cases only one specimen was taken. In one specimen from a case, hemolytic streptococci might be absent but present in a subsequent specimen, which accounts for the absence of some numbers. The last (Arabic) number refers to the strains isolated from the material. Omitted strain numbers are accounted for by loss of cultures or by failure to show sufficient hemolysis on subsequent tests to warrant inclusion in this series.

Hemolysis Tests

The first reaction studied was hemolytic activity. All of our strains were tested by both the plate and tube methods. The pour blood plates were observed according to the directions given by Brown. The changes in the blood plate are given according to his terminology. This reaction is discussed more fully in another paper of this series. The tube method employed was also that of Brown.

As noted above, the primary selection of streptococci as hemolytic was based on the presence of macroscopically visible clearing in the original pour blood plates. On replating the cultures and observing them after forty-eight hours incubation followed by forty-eight hours on ice, it was evident that we were dealing with three types of colonies as regards hemolysis (Brown). The colonies of the alpha type (definitely green and showing only slight if any clearing) were discarded. This type is considered in another article in this series. The remaining cultures fell in either the "alpha prime" or "beta" groups. The "alpha prime" type is characterized by colonies having a distinct zone of clearing, varying in diameter from 2 to 5 mm., at the end of forty-eight hour incubation. When examined under the microscope, the red blood cells are intact immediately around the colony, with the zone of clearing beyond either complete or partial. The "beta" type at the end of forty-eight hours incubation in pour blood plates shows a complete destruction of the red blood cells about the colony and in the area beyond, as determined by microscopic examination. In general the outer edge of the hemolytic zone is more clear cut than in the "alpha prime" types.

The test tube method has added no information. As is evident from the table there is no absolute correlation in the amount of hemolysis in the test tube and the reaction on the blood plate, except that the beta types as a group show a greater ability to hemolyze red blood cells in the test tube than the alpha prime types. The reaction in the blood plate, observed under the conditions advised by Brown, not only gives all the information that the tube method gives but also allows of a further separation into the two fairly distinct varieties (alpha prime and beta). The difference between the two types of colonies may not be very marked in some instances. Additional comparative work as reported in a paper in this series indicates that the slight differences in hemolysis are due essentially to the action of two distinct hemolytic substances. If, as it seems, we are dealing with two different hemolytic substances, that is, with a difference in biologic action in the alpha prime and beta types we are then justified in utilizing this distinction as a primary basis for subdivision.

One group of cultures 168, V, 1, and W, I, 1, 2, 3 and 4 (table 3 and table 7 of the original article) is distinctive.[2] With the blood plate method they give a beta type of hemolysis in that there is a complete hemolysis of the cells about the colony but the surrounding zone is comparatively narrow. With the test tube method these cultures give a slight hemolysis at most and frequently are negative.

Two factors enter probably into this apparent discrepancy. These cultures grow feebly in broth at best and it is probable that, at any one time, the accumulated amount of hemolysin is small because production does not outpace deterioration. In the blood plate, however, the hemolysin acts as it is formed and the resultant hemolysis on the plate is a truer index as to the nature of these strains. More detailed comparative data are given in another article in this series.

The above mentioned cultures, 168, V, 1 and W, I, 1, 2, 3, 4, showed an added peculiarity on the blood agar plate. Their colonies were characterized by a distinctive rosette shape. This characteristic remained constant on further cultivation. Such a colony would be classed by Brown as complex. However, he described no complex colonies of the beta type.

Another peculiar colony morphology was presented by a group of cultures of which serum strain 77 is the prototype. These colonies after twenty-four hours incubation were large, raised, mucoid and glistening; after forty-eight hours they had flattened and dried and when touched with the platinum wire appeared brittle. On further cultivation there was a marked tendency of these colonies to lose this peculiar characteristic. Nine cases yielded colonies which showed this colony morphology. The results of the tests on these strains are summarized in table 8 of the original article.

Fermentation Tests

The next reaction considered was the fermentation of lactose, salicin, mannite, raffinose, saccharose. A highly satisfactory basic medium contained 0.5 per cent. of agar neutral to the andrade indicator (used in a 1 per cent. amount). To this medium was added 1 per cent. of the test substance. This semi-solid medium was inoculated by means of narrow pipette, containing a fresh eighteen hour broth culture. The pipette was thrust down into the medium. and then withdrawn, 0.2 cc. being allowed to flow from the pipette during the withdrawal. The tubes were incubated at 37°C. and observations were made on the first, third and seventh days. The maximum degree of color change and the days on which the maximum color first appeared is recorded in the table.

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[2] See Williams, Unneberg, Goldman and Hussey (11) for original description of these cultures under the terms "Witt" and "Witt like."

In subdividing the strains on the basis of hemolysis and fermentative reactions, Brown's classification has been utilized as far as our data allowed. It will be noted that inulin was not employed. We omitted this carbohydrate because the hemolytic streptococcus ferments it so frequently. This being the case the use of the Brown nomenclature seemed justified notwithstanding the omission of this test.

A moderate degree of variation has been noted in the fermentation tests. This variation has been limited to instances where the avidity for a fermentable substance was not great. That is, a slight positive result might on a successive test be negative or vice versa. In these instances the positive findings were recorded, as it was found that when a large number of tests were made on a given strain more positive results were obtained.

In general the fermentation reactions of the beta types were more constant and they showed a greater fermentative capacity, in that positive reactions were stronger and appeared more promptly. Occasional exceptions were encountered. The most noteworthy example is seen with all of the strains of case 77 and with strains 72 I, 3. The slight reaction with lactose was uniformly elicited and in itself warrants the separation of these cultures from the other pyogenes varieties. Such a peculiarly low but constant avidity for a fermentable substance has apparently a definite classificatory significance among streptococci. As will be noted later these strains are serologically alike. We had encountered this peculiar low avidity for one sugar in cultures obtained during an investigation of an epidemic of septic sore throat. In that instance the epidemic strain was separable from other strains by a low avidity for salicin.

In table 9 of the original article the strains from the same case which showed differences in biological reaction are compiled. The instances where all the strains from the same case are alike in so far as studied, are easily seen in the preceding tables. Comparison shows how relatively few cases yielded dissimilar strains. This is probably no index as to number of varieties present in a single case because the number of strains obtained from each case was relatively few.

DETERMINATION OF FINAL HYDROGEN ION CONCENTRATION IN DEXTROSE BROTH

Avery, and Cullen and Brown have recorded differences between strains of human and of bovine origin in relation to the final hydrogen ion concentration in dextrose broth. The hydrogen ion determination was carried out with our series of cultures in the hope that some differences might be detected. The beef infusion broth was made up with 1 per cent. dextrose, and had a reaction of pH. 7.4 after sterilization. It was inoculated with 0.2 cc. of eighteen hour broth cultures. The colorimetric method was used and readings were made after forty-eight hours incubation. None of the human cultures gave as low a pH reading as most of the bovine controls. The group (W. 168) already mentioned as distinctive with regard to colony morphology and hemolysis, was characterized by giving the lowest pH value among the strains studied.

The number of groups possible among the streptococci on the basis of hemolysis and fermentative reactions, the relatively low case incidence of hemolytic streptococci in the inflammatory conditions and the fact that when present, hemolytic streptococci represent only a very small fraction of the flora, even in the early acute stages, indicates without immunological study that a dominant variety of hemolytic streptococcus was not present among the cases investigated.

AGGLUTINATION TESTS

In the serological investigation of the cultures, the type strains of Dochez, Avery and Lancefield were used to prepare serums for preliminary study. This was done by inoculating sheep with increasing doses of killed cocci followed by injections of live cocci. Antiserums were also prepared by immunizing rabbits with selected cultures of our own series. All injections were given intravenously.

The technic for direct agglutination is given in the original article.

Some cultures when grown in broth were clear or nearly clear due to spontaneous agglutination. In some instances these cultures on thorough shaking, followed by a short period of sedimentation gave a suspension which could be used. With other self agglutinating strains occasional transplants grew without spontaneous agglutination and these could be utilized. However, with quite a number of strains the degree of spontaneous agglutination was so marked and constant that they could not be used at all for agglutination tests. In general these differences as to spontaneous agglutination occurred irregularly without regard to any determinable factors. Many expedients have been tried to prepare uniformly suitable suspensions but without success. The tabulated results, therefore, are the optimum results and give no indication of the repeated attempts and the difficulties encountered.

The results of the direct agglutinations with the serums prepared with the Dochez, Avery and Lancefield strains are given in tables 1 to 8 of the original article. The primary interest is evidently with the piogenes and infrequens groups to which the serum strains belong. In the pyogenes group there are many instances of moderate or marked agglutination by the serums. With some strains this was evident with two or more of the serums. With other strains the action was only marked with one of the serums. Serum 3 agglutinated more strains than any of the other serums.

ABSORPTION TESTS

The next step in the study was the absorption of the serums with the strains agglutinated and also with a certain number of the strains which gave such a marked degree of spontaneous agglutination that no determination of their serological relationship could be made by direct agglutination.

The results of the absorption of serums, D. A. and L, numbers 23, 3, 84, 32 and 273, are given in tables 10 to 14 of the original article. It will be noted that in spite of considerable and often high cross-agglutination, only a very few absorption identities were encountered. The strains of case 122, I (influenza) absorbed the specific agglutinins of serum D, A, L, 32. Strain E (pleurisy) absorbed the agglutinins of serum D, A, L, 23.

Because of the peculiar colony characteristics of the strains from case 77, an anti-serum was prepared for this strain, using rabbits. This serum on direct agglutination reacted with all the strains of case 77 and with some of the other strains which fall fermentatively into the pyogenes group.

The absorption results show that all the strains from case 77 are alike. The other strains acted upon by this serum have not the capacity of absorbing the specific agglutinins. One strain, case 72, I, 3 (the only one which showed the same peculiar fermentative reaction as 77) was also used for absorption, although no direct agglutination reaction could be carried out because of spontaneous agglutination. This strain absorbed the agglutinins from serum 77. We have no data on the colony morphology. If the colony had been characteristic at the time of isolation, this point was overlooked. When the peculiar fermentative reactions of the strain were observed there was nothing characteristic about its colonies but by this time the colonies of serum strain 77 had lost their original peculiarities. The reactions of serum 77 with strains having colony characteristics similar to serum strain 77 but falling in other than the pyogenes group are discussed later.

Strain 18, I (4) and the strains isolated from case 168 with the exception of 168 V 1, fell in the infrequens group and are given in table 2. Only slight reactions were observed with D. A. L. 60 serum. An absorption, carried out with strain 168, I, 1, which gave the highest reaction in this group, resulted negatively. A rabbit serum for strain 168, I, 1 was prepared because of the similarity of its colony morphology to that of strain 77. All of the strains of case 168, with one exception (strain 168, V, 1) are absorptively alike. The one exception also differed from the other strains in its fermentative characteristics.

Six cases yielded cultures which fell in the equi group fermentatively, in that they fermented salicin but failed to ferment lactose, mannite and raffinose. These strains can be divided into two subgroups. Strains from cases 112, 116 and 167 form one group. Strains from case W and strain 168 V, (1) differ from the first group in their hemolytic activity, and in the final hydrogen ion concentration in dextrose broth and in their peculiar colony morphology. Because of these peculiarities an antiserum was prepared (rabbit) for strain W. I, 3 of the second group. This serum agglutinated equally well all the "W" strains, but gave only a slight reaction with strain 168, V, 1 and none with the strains from cases 112, 116, and 167. The cultures of the equi group were not agglutinated by the serums made from strains of the pyogenes group.

The absorption of serum W, 1, 3 showed that the W strains were identical, but that strain 168, V, 1, was different from these. Absorption tests of serum, W, I, 3, with two strains 112 and 116 of the equi group were carried out with negative results. These strains served as negative controls. It is worthy of emphasis that the W strains from pleurisy, approach the bovine type of streptococcus in many of their characteristics.

The strains which fell into the three groups, *Anginosus*, *Salivarius* a and b, and *Mitis* consisted mainly of the alpha prime type. These groups are small and the majority of the cultures were not usable for agglutination tests, because of their extreme spontaneous agglutination. On account of this fact, and in the light of the failure to obtain similarities in the larger groups, the preparation of further serums did not seem indicated. The serums of other groups were tried with alpha prime strains but gave no reactions, which is of interest in comparison with the cross reactions where serum strain and strains tested belong in the same fermentative group.

Colony Peculiarities

The colony distinctiveness of two groups of cultures "like 77" and "like W," have been mentioned previously in this paper. In the summary of the strains with the colony morphology "like *77*" it will be noted that the colony similarity is not paralleled by common fermentative characteristics. The direct agglutinations are given in the tables according to the fermentative groups in which the strains belong. The results of the absorption tests showed that none of the strains which, as far as observed, had this colony morphology were capable of absorbing specific agglutinins from serum *77*. Strain *72*, I, 3 included in table 1 on account of its cultural similarity to strain *77* but whose colony formation had not been studied at the time of isolation is seen to be absorptively identical with strain *77*. As noted under the discussion of the fermentation tests, this strain was the only one encountered which was similar to the strains from case *77* in having peculiarly low avidity for lactose.

The other group having a colony peculiarity is represented by the W strains and strain 168, V, 1. These strains were alike and peculiarly distinctive in all of the cultural and biological characteristics studied. Immunologically the W strains (isolated from the same specimen) are identical but strain 168, V, 1 differs from these. The results obtained with these distinctive groups of streptococci, show very clearly the fallacy of drawing conclusions as to the identity of strains even where the colony morphology and also all the other observed biologically characteristics are alike. If then, in a series of cases of respiratory inflammations, there is found such a distinctive type, its presence is only suggestive as regards probable etiology. No conclusions can be drawn as to the identity of such strains until a serological investigation including agglutinin absorption has been made. Without such investigation, the emphasis placed by some investigators upon the frequent isolation from influenza cases of a streptococcus having distinctive cultural characteristics is wholly unjustified.

A further study of the strains isolated from the various cases noted did not seem indicated. As the organisms were studied the tendency was toward the separation of groups, the number of which increased in proportion to the number of characteristics studied, and the prospect of encountering a dominant type became less and less promising. Moreover, the possibility of further serological investigation was limited by the extreme spontaneous agglutination of some of the cultures. The relatively low incidence of streptococcus hemolyticus and the relatively small numbers present when they did occur in inflammations of the mucous membranes also gave little indication that this organism was a common factor in any appreciable number of the cases investigated. Not only is there no evidence of a distinctive strain among the cases as a whole but likewise no indication of a distinctive strain common to any one of the types of disease studied.

Conclusions

A study of the streptococci of the hemolytic group obtained from inflammations of the respiratory tract shows that these strains fall into many cultural groups and sub-groups. A serological study showed so few similarities that the probability of a dominant strain seemed remote. There was no correlation between the grouping and the type of disease. These observations and the relative infrequence of streptococci of the hemolytic group in inflammations of the respiratory tract seem to justify the conclusion that none of the strains isolated were of primary etiological importance.

For bibliography see the original article.

STUDIES ON ACUTE RESPIRATORY INFECTIONS[1]

Differences in the Character of the Hemolytic Action of Streptococci and the Relative Value of Various Methods of Demonstrating These Differences

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Lucy Mishulow

Differences in the hemolytic activity of streptococci when grown on blood agar were noted by Schottmuller in 1903. The blood-agar plate was generally employed to determine the hemolytic ability of streptococci until Lyall (1914) advised the use of the tube method as a means of differentiation. Later Smith and Brown, using the blood agar plate method, introduced a new nomenclature to indicate the reactions obtained. Brown, elaborated these findings and gave detailed descriptions of the characteristics to be observed and advocated a classification based upon such observations. He gave a very complete review of the whole subject.

The superiority of either the plate method or the tube method depends upon the answers to the following questions: (a) Does the plate method bring out all the differential characteristics not shown by the latter method, or is the reverse true? (b) Does each method bring out certain characteristics? (c) Is there with either method a greater correlation between the results elicited and any determinable differences in the substances producing hemolysis?

The observations in this paper are offered in answer to these questions.

The streptococcus cultures employed were forty-two strains from the series reported by Valentine and Mishulow.

	Strains
Naso-pharynx, inflamed or "normal"	27
Pleurisy, purulent	4
Mastoiditis, blood culture	2
Tonsilitis	1
Serological Types of Dochez, Avery and Lancefield (6)	6
Brown (7) bovine and human "Types"	2

The strains were kept on blood streaked agar slants. Fresh transfers were made before each experiment.

The method of demonstrating hemolysis and the conditions of observation were as follows:

Blood plates: Two loops of an eighteen to twenty hour broth culture were transferred to a tube of sterile broth which was shaken thoroughly and two loops of this dilution were inoculated into 1 cubic centimeter of citrated horse blood. Ten cubic centimeters of liquid veal-agar were added and the mixture then poured into a Petri dish. By this procedure 10 to 25 colonies were obtained and the well separated colonies could be studied in detail. The plates were observed after twenty-four and forty-eight hours incubation at 37°C. They were then placed in the refrigerator for forty-eight hours and again observed.

The results recorded in a table are those noted after forty-eight hours in the incubator. With the cultures studied, forty-eight hours of refrigeration did not bring out any additional differential points.

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[1] This study is part of an investigation on acute respiratory diseases carried on under a grant from the Influenza Commission Fund of the Metropolitan Life Insurance Company. This series of studies was carried out under the direction of William H. Park, Anna W. Williams and Charles Krumwiede.

Tube hemolysis. (Lyall method.) The red cells from fresh defibrinated horse-blood were washed three times in 0.85 per cent. saline, and a 5 per cent. suspension in saline was made. To 1 cc. of this suspension fo red blood cells was added 0.5 cc. of an eighteen to twenty hour broth culture (beef infusion, peptone 1 per cent., NaCl 0.5 per cent., reaction pH 7.8 to 7.9), and after the tubes were shaken the mixtures were placed in a water-bath at 37°C. for two hours and were again shaken every half hour to prevent sedimentation of the blood cells. After this incubation, all tubes in which hemolysis was not complete were rapidly centrifuged to facilitate observation of the degree of hemolysis.

Brown's modification of the tube method. An eighteen to twenty hour broth culture was diluted 1 to 10 and 1 to 20 with physiological salt solution. To 1 c.c. of each dilution was added 1 drop of sterile defibrinated horse-blood and the mixtures were incubated in a water bath at 37°C. for two hours. Readings were made in the same manner as by Lyall's tube method. The proportion of red blood cells in different samples of defibrinated horse-blood varied considerably. To obtain uniformity in the amount of red cells used in the successive tests, the defibrinated blood was centrifuged and serum was added or removed until the volume of cells was to the volume of serum as 1 is to 2. The same broth cultures incubated at 37°C. for eighteen to twenty hours were used in making the hemolytic tests by the three methods described.

In tabulating the character of the hemolysis on the blood plates Smith and Brown's and Brown's nomenclature is employed. The strains that gave clear colorless zones of hemolysis in forty-eight hours with microscopically complete destruction of red blood cells around each colony were designated *beta*. Those strains that produced hemolysis macroscopically similar to the above, but the colonies of which on microscopic examination were found surrounded by blood cells, were termed *alpha* prime. The strains which produced no definite hemolysis after forty-eight hours incubation, but definite although usually narrow zones of clearing after forty-eight hours refrigeration, were termed *alpha*. The last group usually showed green discolorations in and about the colonies.

The tabulation of the results with the plate method (see original article, Table I), shows that of the 42 strains 32 gave the *beta* type hemolysis, 6 the *alpha* prime, and 4 the *alpha* type.

The question was raised in a few instances as to whether a strain should be classified as a *beta* or an *alpha* prime type. Most of these doubtful strains were finally considered *alpha* prime types because cells, although few in number and partly disintegrated, were still discernable about the colonies.

Two strains, 168 II(1) and 82 (1), gave wide clearcut zones of hemolysis, without any sign of red cells about the colonies. In the outer zone, however, there were visible on microscopic examination a few partly disintegrated cells. These strains were classified as *beta* types.

Five strains (168V (1), 10X, 11X, 12X and 13X) caused only slight hemolysis on the blood plate after twenty-four hours. After forty-eight hours there was a well defined zone of hemolysis and on microscopic examination no red cells were found about the colonies. Strain 168 V (1) was slower in its hemolytic activity than the other four strains mentioned. In retests this strain occassionally showed no hemolysis after twenty-four hours. All of the five strains because of the disappearance of the red cells about the colony were classed as *beta* types.

The results obtained with the tube methods, compared with each other and with the type of hemolysis on the blood agar plate (table 1), are summarized as follows:

Beta type 32; with Lyall method = 32 positive, 0 negative.
Alpha prime type 6; with Lyall method = 6 positive, 0 negative.
Alpha type 4; with Lyall method = 1 positive, 3 negative.
Beta type 32; with Brown method = 26 positive, 6 negative.
Alpha prime type 6; with Brown method = 4 positive, 2 negative.
Alpha type 4; with Brown method = 0 positive, 4 negative.

The six *beta* strains (10X, 11X, 12X, 13X, 168 V (1) and B cow 18) which failed to hemolyse by the Brown method gave variable amount of hemolysis by the Lyall method when retested. As noted above, five of these cultures (human origin) were also slower in producing hemolysis when grown on blood agar. These strains of human origin were characterized by further peculiarities. As is noted in table 1, the pH figure after growth in 1 per cent dextrose broth is the lowest for this group of strains. This figure pH 4.6 or 4.7 is usually indicative of bovine origin (Avery and Cullen; Brown. This is the figure given by strain B cow 18 (bovine origin).

These five human strains grew slowly and the amount of growth was never heavy. The failure to hemolyse regularly by the Lyall method may be due to the two factors; namely, the small amount of hemolysin present at any one time due to scant growth and the progressive deterioration of hemolysin in fluid media. The amount produced minus the amount deteriorated might be so small that either no reaction resulted or at most a slight reaction. When this amount of hemolysin was reduced by dilution (Brown's method) no reaction resulted. The hemolysis on the plate although slow was always well defined after forty-eight hours. The more regular end results obtained with the plate may be due to the traces of hemolysin which acted directly upon the cells as fast as formed, thus eliminating to a large extent a loss of effect due to deterioration. If this explanation is valid, the plate method reveals the effect of all or nearly all the hemolysin formed, whereas the tube method reveals only the effect of that which has not deteriorated.

It appears, therefore, that for the demonstration of hemolysis by streptococci the blood plate method is more reliable than the test-tube methods because a certain percentage of feebly growing streptococci may fail to show hemolysis in the test-tube. The plate method is also more valuable for preliminary classification because it brings out differences in hemolysis that the tube methods fail to show. Since the completion of this work Clawson has published similar conclusions with regard to the value of the blood plate. The Brown modification of the tube method has not been of value with the human strains reported in this paper. This method was used by Brown primarily for the differentiation of streptococci of bovine origin.

The failure of the tube method to differentiate between the *beta* and *alpha* types, which were differentiable by the plate method, raised the question as to whether the mechanism of hemolysis might not be different with these two varieties. If an essential difference could be demonstrated, this would be added evidence of the value of the plate method as a means of preliminary differentiation.

The following experiments were designed primarily to determine whether hemolytic action might be due to an extracellar substance in the one case and an intracellular substance in the other.

An eighteen to twenty hour broth culture was shaken by hand to distribute the cocci evenly. One cc. of washed 5 per cent. horse-blood was added to 0.5 cc. of a sample of the broth culture (Lyall's method) and the mixture was incubated in a water bath at 37°C. for two hours, as a control. The remainder of the culture was rapidly centrifuged and the sedimented bacteria were suspended in distilled water for extraction. This suspension was made slightly more concentrated than the original broth culture and was divided into two parts. One sample (C) was placed in the ice-box and the other was shaken in a mechanical shaker for two and a half hours. A portion of the shaken sample (A) was tested immediately after shaking and the remainder (B) after further extraction in the icebox overnight. To obtain the extracts, all of the suspensions were centrifuged and to the clear extract was added 1/10 its volume of 9.0 per cent. sodium chloride to make it isotonic. The sedimented bacteria from each of the three extracts were resuspended in physiological saline and were also tested separately in portions A′, B′ C′. In each instance 1 cc. of a 5 per cent. suspension of washed red blood cells (horse) was added to 0.5 cc. of the test substance (extract or suspension of cocci after extraction) to determine the hemolytic activity.

The results are given in table 2 (see original article) and show that the extracts (refrigerator) of the *alpha* types had hemolytic action which was as strong as the original broth culture, or stronger. Extraction by shaking tended to destroy the hemolysin. Culture D.A.L. No. 32 (*beta* type), however, showed strong hemolysis immediately after shaking, which decreased after refrigeration. This result as well as that of extraction by refrigeration without shaking indicates that the hemolysin of this strain is more labile with regard to time than that of the *alpha* types.

The hemolytic activity possessed by the suspensions of the extracted cocci was relatively the same as with the original broth culture. The question whether this hemolysis was due to preformed substances or to the products of growth during the test remains unanswered.

No special study was made of methemoglobin production but as far as observed there was a sharp difference depending on whether extracts or suspensions of cocci were used; the former gave negative and the latter positive results when tested before refrigeration.

The question as to the persistence of the hemolytic activity of broth cultures of the various types was next considered. Broth cultures were made and the whole culture as well as the clear broth obtained by centrifugation were tested for hemolytic activity after incubation of the cultures for one, two, three, four and six days respectively, using Lyall's tube method (table 3 of the original article).

The *beta* and *alpha* types could not be differentiated by this method when the whole culture was used. With the supernatant fluid, however, there was a distinct difference. The *beta* types showed an average greater hemolytic activity after twenty-four hours incubation, which disappeared on further incubation. On the other hand the *alpha* types showed an increased hemolytic activity on longer incubation (see table 3). This seems to indicate that the hemolytic action of the *beta* type is essentially due to an extracellular product, labile in character; whereas that of the *alpha* types is due to the direct action of the cocci or of the products of their autolysis. The methemoglobin reactions were irregular, but it is noteworthy that they were consistently weak with the clarified broth.

The resistance to heat of the substances in cultures which cause hemolysis and the methemoglobin production was also investigated (Lyall tube method). The results of these experiments are given in table 4 (see original article).

There was a marked difference in the results obtained with the *beta* and the *alpha* types in regard to this point. With both *alpha* types there was a disappearance of hemolytic activity after heating at 55°C. for five minutes, while the *beta* hemolysin, resisted heating for five to ten minutes. These results apparently correlate and strengthen the deduction drawn from the previous experiments. Again, the indications are that the cultures of the *beta* type contain a preformed and least partially free hemolysin which resists heating for five to ten minutes. The hemolytic activity of the *alpha* types is apparently due to direct action of the cocci, or of substances liberated by autolysis. The heating for five minutes had probably killed a large number of cocci or inhibited their activity. As previous experiments indicate, the autolytic products had probably not developed in appreciable amounts during the eighteen hours incubation. The result of both these factors was an absence of hemolysis.

The results obtained in these studies probably explain to some extent the conflicting opinions of previous workers as to whether or not the hemolytic activity of streptococci is due primarily to an extracellular substance. The varying results obtained by different workers may depend on the types of streptococci studied and the methods used for investigation. De Kruif in a review of work of this nature has shown very clearly that with his cultures hemolysin contained in the clear broth reached a maximum concentration and then rather rapidly disappeared. The whole culture, however, retained its hemolytic capacity for a much longer time than the clear broth. These results correspond with the results of the filtration experiments of Meader and Robinson. They found that when a broth culture was rapidly filtered there was considerable hemolysin in the filtrate; whereas with slow filtration the filtrate gave negative results.

The conclusion that the *alpha types* owe their hemolytic activity primarily to direct action of the cocci brings up the question as to the mechanism of this action. With both the *beta* and the *alpha* types there are other factors involved besides the mere liberation of the hemoglobin. This is plainly evident with the blood plate method, where hemolysis, is accompanied by the development of a clear transparent decolorized area. The action of bacteria upon the stroma and hemoglobin together with the mechanism of this action has been investigated by several workers. We have not considered this aspect of the problem but have limited our efforts to the demonstration of primary differences in hemolytic action that would correlate with the blood plate picture, which depends not only upon simple hemolysis but upon the activity of other factors as well.

Conclusions

The blood agar plate, incubated for forty-eight hours, is a more reliable method of determining the hemolytic action of streptococci than the tube method, because differences in hemolytic action can be observed with the blood agar plate which are not observable with the tube method.

The differences in hemolysis observed in the plate are correlated with primary differences in the mechanism of hemolysis. The hemolytic action of the *beta* type is primarily due to a hemolysin which is a soluble extracellar product. The hemolytic activity of the *alpha* types seems to be due to the direct action of the cocci or to products liberated by autolysis. The plate method which brings out these points, is, therefore, the more valid method on which to base a classification.

A modification of the tube method, in which only the clarified broth is used for the test, appears to be an improvement on the regular method. However, it may fail in some instances to reveal hemolytic action where the plate method would give positive results.

For bibliography see the original article.

STUDIES ON ACUTE RESPIRATORY INFECTIONS[1]

The Nature and Value of a So-Called Precipitin Reaction as Applied to The Serological Grouping of Streptococci

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CHARLES KRUMWIEDE AND EUGENIA VALENTINE

Barnes, in a study on the cultural and serological relationship of hemolytic streptococci reported recently that the serums of rabbits intensively immunized against the streptococcus will give precipitin reactions with streptococcus antigens even when such serums are diluted as high as 1-3200.

His method of eliciting what he terms a precipitin reaction, was as follows: The streptococci were grown in broth for forty-eight hours at 37°C. The broth cultures were centrifuged at high speed to sediment the cocci. In each test a constant amount (1 cc.) of clear undiluted supernatant broth antigen was added to an equal amount of the diluted antistreptococcus serum. These mixtures as well as controls with normal rabbit serum and with uninoculated broth were incubated at 37°C. for eight to ten hours, removed to the ice-box for two hours and then read. Sterility tests were made to eliminate the possibility of the precipitate being bacterial growth.

To us the results as reported by Barnes seemed most extraordinary. In our experience the most potent serums would when added to concentrated antigens, give precipitin reactions only in dilutions as high as 1:100 or 1:200 at most. Even the prolonged incubation period used by Barnes did not seem to us to explain his positive results with thousand-fold and greater dilutions of serum.

It seemed advisable, therefore, to investigate the reaction obtained by Barnes to determine whether his interpretation of its nature was correct. If the reaction were actually a precipitin reaction the relative specificity of the results would be open to question; in addition the applicability of the antibody-absorption method would be limited, because the absorption of precipitins gives, not infrequently, non-specific results. Even if Barnes' interpretation were not correct, the reaction might have an advantage over the regular agglutination method, by eliminating the all too frequently disturbing factor of spontaneous agglutination.

Our experiences with the method described by Barnes when controlled by the regular agglutination methods are not given here in full. The table in the original article sufficiently illustrates our findings. These results also suffice to show the nature of the reaction.

The striking point in the table is the difference in the reactions obtained with the method described by Barnes when parallel tests were incubated at 37°C. and at 55°C. respectively. At 55°C. there is seen a reduction of the degree of reaction within the limits to be expected with the precipitin reaction and which we actually obtained with culture filtrate antigens. If the reaction described by Barnes were a true precipitin reaction there should not be this difference at the two temperatures employed.

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[1] One of a series of studies carried out under the direction of William H. Park, Anna W. Williams and Charles Krumwiede. The previous studies in this series were reported in this Journal, 1921, vol. 6, no. 1. This investigation was made possible by a grant of money from the Metropolitan Life Insurance Influenza Fund for a part of the expense.

Numerous tubes in both series were used for bacteriological tests. The contents of each tube was added to a separate tube of blood agar and the mixtures poured into petri dishes. After incubation, abundant growth was obtained from all the samples of the 37°C. series but from none of the 55°C. series. Likewise, smear and hanging drop examinations of the sediment of centrifuged tubes of similar series incubated at 37°C., showed the presence of numerous streptococci. Where clumps were visible to the naked eye, clumps of streptococci were also seen in the hanging drop preparations. The constant evidences of growth even from the saline control in every series incubated at 37°C. and the absence of such growth in the 55°C. series, absolutely contradicts Barnes' claim that the precipitate is not due to bacterial growth.

After we had obtained the above results, two statements in Barnes' article seemed very significant. He found that the addition of cresol 0.3 per cent "seriously interfered with the precipitin reaction." Likewise, he found that the supernatant broth from a culture gave "better results" than an extract from killed cocci. How much reaction was obtained and in what dilution, is not stated in regard to these extracts.

There is only one possible conclusion as to the mechanism of the reaction described by Barnes. Although with the immune serums there is some true precipitin action in the lower dilutions, the essential phenomenon is that of growth and agglutination of the cocci.

As to the value of the reaction it is evident from a study of the table that with cultures showing little tendency to spontaneous agglutination, the results were about the same as those obtained with the ordinary agglutination method. With cultures showing a marked tendency to spontaneous agglutination, no better results were obtained with the miscalled precipitin method which for convenience we shall term the growth-agglutination method. To determine the degree of spontaneous agglutination a comparison, dilution by dilution, of the results obtained with the immune and with the normal serum must be made. In the lowest dilutions the serum present may limit spontaneous agglutination because in these dilutions the cocci are growing in a medium qualitatively like serum broth. In the highest dilutions the amount of growth decreases and consequently the evidences of spontaneous agglutination become less marked.

In a few instances given in the table, the results have been somewhat better with the growth agglutination method than with ordinary agglutination. Thus with serum 3 and strain 3 the contrast between normal and immune serum is more marked. With the same serum and strains 23 and 171, the degree of group agglutination is less marked.

These results indicate that the application of the method might be attempted if the possibility of obtaining results was sufficiently important to overbalance its drawbacks, namely—the frequent failures due to spontaneous agglutination, the time for centrifuging, the excessive care necessary to prevent contamination and the difficulty of reading the relatively less marked reactions obtained. If this method were employed, the question would be raised immediately as to the applicability of the absorption method to evaluate the reactions obtained.

It does not seem of value to tabulate our absorption tests. As was to be expected from the data given in table 1 the results obtained were practically the same as with the regular agglutinin absorption method. Where spontaneous agglutination interfered with one method it interfered with the other. Somewhat greater difficulty was encountered with the growth method, as in some instances a larger absorbing dose was necessary than was the case with the regular agglutinin absorption method. In carrying out these absorptions the mixtures of serum and absorbing cocci were incubated at 55°C. to prevent growth of the absorbing strain in the subsequent tests. This method was satisfactory as was shown by the parallel tests with the regular agglutination method.

Conclusion

The method described by Barnes is not, as he concluded, a precipitin reaction. The reaction is due to the growth and agglutination of the cocci in the mixtures of broth and diluted serum. The results obtained by this method are essentially the same as those obtained by the ordinary agglutination method.

The method is of no especial value as regards the elimination of the factor of spontaneous agglutination which renders so difficult the serological investigation of streptococci.

For bibliography see the original article.

A PECULIAR FAILURE OF HEMOLYTIC ACTION IN A STRAIN OF HEMOLYTIC STREPTOCOCCUS

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Eugenia Valentine

While titrating by the tube method the hemolytic capacity of a series of streptococcus cultures of the *beta* hemolytic type, a peculiar absence of hemolysis was observed with one culture.

With the strain W 12, 0.5 cc. of an 18-hour broth culture failed to give hemolysis when mixed with 1 cc. of 5 per cent. washed red cell (horse), while 0.4 cc. of the culture plus 0.1 cc. of sterile broth resulted in almost complete hemolysis of the red cells.

Another strain having the same characteristics as W 12, but differing from it agglutinatively, also showed hemolysis in all the tubes.

The failure of the whole culture of strain W 12 to lake red cells until the addition of sterile broth, is of interest from the practical standpoint, for it may be a source of error in testing the hemolytic activity by the tube method usually employed. From the theoretical standpoint it seemed of interest to explain, if possible, the mechanism of this phenomenon.

In most of the further experiments, 0.5 cc. and 0.2 cc. of culture were used. The phenomenon was observed with marked constancy without regard to the kind of broth employed, that is, whether made of veal or of beef, the mode of sterilization, the period of storage and the variations of reaction within the limits suitable for growth.

It was found, however, that 0.5 cc. of a broth culture of W 12, which had been incubated for only six hours, caused hemolysis, without the addition of fresh broth, but that the same culture incubated for fifteen or eighteen hours did not. The failure of hemolysis by the 18-hour culture was always prevented by the addition of 0.1 cc. of sterile broth, and the addition of as little as 0.03 cc. of broth resulted in the development of some hemolysis.

When the clarified supernatant broth of an 18-hour culture of W 12 was used in place of sterile broth, no hemolysis took place. Heating this supernatant broth to 56°C. and 100°C. for fifteen minutes gave no different results. The supernatant broth from an 18-hour culture when added to a 6-hour culture did not prevent hemolysis. Adding peptone water to 18-hour broth cultures was as effectual in renewing hemolytic activity as the addition of sterile broth. Sterile horse serum, however, did not serve so well. Acid production was evidently not a factor, for neutralization of the small amount of acid produced did not result in hemolysis.

The outstanding points were therefore: the active hemolysis after six hours' incubation, the absence of hemolysis after long incubation, the renewal of hemolytic action by the addition of broth or peptone water, that is, a fresh food supply. This raised the question: Was the failure of hemolysis due to the cessation of growth and was the resumption of hemolytic action, after the addition of fresh broth, due to renewed multiplication?

This point was investigated by making poured plates. After 2 hours' incubation of the tube to which no fresh broth had been added, plate counts showed at most a slight increase in the number of cocci. The tube, however, to which fresh broth had been added, showed after two hours' incubation an increase of 30 to 60 per cent. in the number of colonies developing in the plates.

SUMMARY

It would seem that the failure of hemolysis of the strain of hemolytic streptococcus studied was due to cessation of growth which was renewed by the addition of a small amount of fresh broth. The reason, however, why this culture should present this peculiarity remains obscure.

For bibliography see the original article.

THE LOSS OF HEMOLYTIC CAPACITY BY A FRACTION OF A CULTURE OF A HEMOLYTIC STREPTOCOCCUS WITHOUT CHANGE IN AGGLUTINATION CHARACTERISTICS.

Published in The Journal of Experimental Medicine, 1922, Vol. XXXVI, pp. 157-162

By Eugenia Valentine and Charles Krumwiede

The partial or complete loss by the hemolytic streptococcus of its ability to hemolyze red blood cells has been recorded. In the studies on variations of streptococci as regards hemolysis and other characteristics, no complete and adequately controlled observations on the agglutination, agglutinin-binding, and agglutinogenic characteristics have been made, as far as we know, to determine whether variation in function has been associated with change in the antigenic constituents of the cocci. We are reporting, therefore, comparative observations on a strain of hemolytic streptococcus which has developed a non-hemolytic fraction.

The strain under consideration is one of the Dochez, Avery, and Lancefield type strains, No. 32, which had been isolated from the lung at autopsy (bronchopneumonia following measles).

Described according to the recommendations of Smith and Brown and Brown, the hemolytic fraction has all the characteristics of the *beta* types with a 4 to 5 mm. transparent zone of hemolysis. The green fraction is an *alpha* type with no visible clearing except for the late development of a very narrow green translucent zone, which microscopically shows scarcely any diminution in the number of red cells. Immediately surrounding the colony there is no reduction of the number of cells. The surface colonies of the green type fraction are moist, green-zoned, and resemble closely a typical pneumococcus colony.

The hemolytic properties of the two fractions were also tested with the blood tube method (Lyall). With the *beta* type, hemolysis was prompt; 0.3 cc. of a 15-hour broth culture caused complete hemolysis, and as little as 0.05 cc. caused partial hemoylsis. The green fraction showed no hemolysis with 0.5 cc.; the color of the red cells was changed to a deep magneta, but not to the typical brown shown by the pneumococcus used as a control.

Morphologically and culturally the two fractions show no differences, other than those noted above.

Agglutination and Agglutinin Absorption Tests.

Agglutination tests were set up with the serum of a sheep immunized against the original Strain 32. Fortunately all fishings of the hemolytic and green fractions grew with a diffuse cloud in beef broth so there was no difficulty in obtaining satisfactory agglutination results. The results of the test with the sheep serum showed that both fractions were agglutinated equally well, and to the titer of the serum strain, No. 32. Likewise the absorption of this serum by either fraction removed completely the agglutinins for both fractions and for the serum strain, No. 32.

For more conclusive evidence rabbits were immunized with a representative of each of the fractions. At each injection control plates were made to check the character of the injected cocci. The serums obtained were used for agglutination and agglutinin absorption tests.

Direct agglutination and agglutinin absorption tests, with the anti-serums for both the hemolytic and green fractions, were also carried out on the original Strain 32, and on the other fishings, H.2, H.3, and G.2, G.3 isolated at the time the cleavage was noted. The reactions of these strains were identical with those of the serum strains H.1 and G.1 given in Table I of the original article.

The ability of either fraction to absorb the agglutinins from the anti-serum of the other fraction shows that the loss of hemolytic power has not been accompanied by any change in the agglutinogenic complex of the cocci.

VIRULENCE TESTS.

The virulence of the two strains was tested by mouse inoculation with 18 hour broth cultures. With the hemolytic variety of 0.5 cc. killed uniformly, 0.25 cc. irregularly. The green type was less virulent, 1 to 1.5 cc. causing death, but only irregularly.

Attempts were made to increase the virulence of the green variant by mouse passage in the hope that the hemolytic capacity might return with the increase in virulence. After fourteen consecutive passages the virulence was raised so that $\frac{1}{60}$ cc. caused death. This increased virulence was not associated with the development of hemolytic colonies. The mouse passage caused no marked fall in the agglutinability and no change in the absorptive capacity of the culture. A further attempt to restore the hemolytic capacity to the green variant was made by growing it in blood broth, but twenty-five successive transfers in this medium have resulted in no change. The hemolytic fraction has also bred true after twenty-five blood broth generations.

Replating of the original Strain 32 showed the persistence of the two varieties throughout August, 1921. At that time fresh isolations of the two fractions were made and found to be identical culturally and serologically with the previous isolations. Since then, two platings of the original strain have failed to show any green colonies although a large number of plates were made.

TESTS WITH OTHER STRAINS.

Twenty other strains of hemolytic streptococcus, the majority from pathological conditions, which had been under cultivation for from 2 to 8 years, were tested in order to determine whether or not any loss of hemolysis had occurred. Frequent platings of these strains have shown no change in the hemolytic capacity, as all colonies give well marked zones. The observations are in contrast with those of Clawson who found that out of 116 strains of hemolytic streptococcus under cultivation for 1 to 4 years, 54 showed one or more green colonies when plated in blood agar. He tested the constancy of substrains obtained from six cultures. His substrains showed little tendency to breed true, the green substrains in all instances showing reversions to the hemolytic type.

CONCLUSIONS.

A culture of hemolytic streptococcus developed a non-hemolytic fraction which as determined by its agglutinogenic, agglutination, and agglutinin absorption characters was identical with the hemolytic fraction, the only variation being one of function, or physiological action. This functional variation has proved permanent, as long as observed, for both fractions have continued to breed true.

This study is offered as additional evidence in favor of the hypothesis that functional changes among bacteria are, at most, only very infrequently associated with changes in the antigenic matrix of bacteria.

OBSERVATIONS ON THE CROSS PROTECTIVE POWER OF ANTI-PNEUMOCOCCUS MONOVALENT SERA, TYPES I, II AND III[1]

With a Note on the Cross Protection Afforded by So-called "Antibody Solution"

Published in The Journal of Immunology, 1924, Vol. IX, pp. 505-513

Betty S. Kolchin and Louis Gross

In 1922 Cecil and Larsen published a paper dealing with their observations on the results of treating pneumococcus pneumonia with Huntoon's Types I, II and III polyvalent antibody solution[2] where they brought out among other things the suggestion that the statistical results indicated that the mortality rate from group IV pneumococcus pneumonia was lowered by such treatment. Similar observations were reported by Conner in the same year.

This result was due either to an accidental fluctuation in the mortality rate of the treated and control cases or to genuine antibody protection against Type IV organisms by an antipneumococcus serum made from the three fixed pneumococcus Types I, II and III. This question of possible cross protection was considered by the Influenza Commission of the Metropolitan Life Insurance Society of great interest and practical importance. This investigation was undertaken by the writers at the suggestion of the Commission.

If it were true that considerable cross protection against group IV pneumococcus strains could be obtained by combined Type I-II-III antibodies, this would represent an encouraging step in the direction of serum treatment of the pneumonias due to this heterogeneous group.

Our first step was to try out cross protection in mice against group IV strains. In an attempt to confirm the clinical findings referred to above, we employed at first Huntoon's antibody solution.

We accordingly obtained fifty-one strains believed to belong to group IV which had been isolated from pneumonia cases in several large hospitals.

These strains were subjected by us to the agglutination test and all but three were confirmed as belonging to group IV.

Two of the three mentioned agglutinated typically with serum Type I, and the third one seemed to be a mixture of Type I and Type IV. In the latter case, after passages through mice the Type I strain seemed to have outgrown the Type IV strain and agglutinated typically with Type I serum. This admixture was also confirmed by making numerous fishings from the original culture and by subsequently agglutinating them. These agglutinations established the presence of two mixed strains in the original culture, a Type I strain and Type IV strain. These findings suggested that about 5 per cent. of identifications by routine typing are erroneous.

This report deals with the 48 remaining strains. The virulence of these strains was determined by injecting intraperitoneally graded doses of an eighteen-hour blood broth culture into mice weighing 18.5 to 21 grams. The graded doses were made up to 0.5 cc. volume with broth, and the smalles doses as expressed in fractions of 1 cc. of the original culture which killed within five days, was taken as indicating the virulence of the strain. Bacterial counts of these doses were determined by plating measured amounts of the culture in blood agar pour plates.

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[1] This investigation was aided by a grant from the Influenza Commission of the Metropolitan Life Insurance Society.

[2] Huntoon's polyvalent antibody solution is prepared by agglutinating heavy emulsions of the types, I, II and III pneumococci in polyvalent antipneumococcus sera with subsequent centrifugalization of the organisms and dissociation of the antibodies in weak alkaline solution.

If the virulence was less than such as would kill a mouse within five days after an injection of 0.00001 cc. the strain was subjected to a process of raising its virulence. For this purpose, we passed the strain through mice and tested the virulence after approximately ten passages.

It might not be out of place to mention here a point in the technic of mouse to mouse transfer of the pneumococcus strain, which we found very helpful. After intraperitoneal injection the mouse was usually found dead the next day. The dead mouse was pinned to a board and its fur soaked in 5 per cent. carbolic acid. With sterile scissors the fur was snipped along the sides and across the lower abdomen of the mouse. Grasping the lower border of the flap of fur thus freed, by means of forceps, it was torn over the head. This exposed the abdominal and chest wall untouched. The ribs were then cut through with sterile scissors, and removed. Almost invariably an abundant pleural exudate rich in organisms and free from contamination was found. This was sucked up into a sterile syringe and injected into a fresh mouse. The advantage of this method over that of sucking up the peritoneal material at the site of the previous injection lies in the fact, that the pleural exudate possibly contains the more virulent organisms which apparently passed through the blood system.

Agglutinating sera were prepared against the original strains as well as against the corresponding strains after the passages through the mice. The object of these sera was to determine whether the final strain obtained after the last mouse passage was identical with the original, and if any strains of group IV were found against which protection could be afforded, whether there was any serological relationship among them.

Of the 48 group IV strains which we obtained, 26 were ultimately of such a virulence that a mouse weighing 18.5 to 21 grams was killed within five days with doses of 0.00001 cc. or less. Thirteen of these strains were originally of a sufficiently high virulence and 13 had to be raised. Eleven strains showed no appreciable increase in virulence after 10 to 15 passages through mice, but were nevertheless tested for protection by the antibody solution. Eleven strains were so low in original virulence that no effort was made to raise it. These were discarded.

In the protection tests with the Huntoon antibody solution, the usual procedure was adopted. One-half cubic centimeter doses containing graded amounts of an eighteen-hour blood broth culture of a strain from group IV penumococcus were mixed with 0.5 cc. of a 40 per cent. solution of the Huntoon polyvalent antibody[3] and injected into a series of mice. The mice averaged 18.5 to 21 grams in weight and death within five days after injection was considered as standard. In this way we were able to pick out from among the 37 tested strains, three against which protection up to 1000 fatal doses was obtained. Even though two subsequent repetitions failed to confirm this protection, the apparent discovery of these three strains led us to further work with several interesting observations. We shall therefore have

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[3] The dilutions of blood broth cultures as well as of the antibody solution were made up with plain broth.

TABLE 1

PROTECTION TESTS AGAINST CULTURES OF FIXED TYPES I, II and III PNEUMOCOCCI WITH HUNTOON'S POLYVALENT ANTIBODY SOLUTION

DOSES OF CULTURE IN FRACTIONS OF 1 CC.	TYPE I PNEUMOCOCCUS		TYPE II PNEUMOCOCCUS		TYPE III PNEUMOCOCCUS	
	Huntoon's solution	Virulence control	Huntoon's solution	Virulence control	Huntoon's solution	Virulence control
1.0	Dead		Dead			
0.5	Dead		Dead			
0.1	Dead		Dead			
0.01	Living		Dead			
0.001	Living		Dead			
0.000,1	Living		Living		Dead	
0.000,01	Living		Living		Dead	
0.000,001	Living		Living		Dead	
0.000,000,1	Living	Dead	Living		Dead	Dead
0.000,000,01	Living	Dead	Living	Dead	Dead	Dead
0.000,000,001	Living	Dead	Living	Dead	Not done	Not done
Protection obtained against	10,000,000 doses		100,000 doses		No protection	

occasion to refer to these strains again. For the sake of brevity we shall refer to these as the "three strains."

The identity of the "three strains" with the corresponding original ones was checked up by means of agglutination and cross agglutination tests.

Under the belief that these "three strains" were genuinely protected and before we had obtained the results from the repeated protection tests, we

TABLE 2

PROTECTION TESTS WITH TYPE I HORSE SERUM AGAINST CULTURES OF FIXED TYPES I, II AND III

Anti-pneumococcus horse serum Type I

DOSES OF CULTURES IN FRACTIONS OF 1 CC.	PNEUMOCOCCUS TYPE I	VIRULENCE CONTROL	PNEUMOCOCCUS TYPE II	VIRULENCE CONTROL	PNEUMOCOCCUS TYPE III	VIRULENCE CONTROL
0.5	Living		Dead		Dead	
0.1	Dead		Dead		Dead	
0.01	Living		Dead		Dead	
0.001	Living		Dead		Dead	
0.000,1	Living	Dead	Dead	Dead	Dead	Dead
0.000,01	Living	Dead	Dead	Dead	Living	Dead
0.000,001	Living	Dead	Dead	Dead	Living	Dead
0.000,000,1	Living	Sick	Living	Dead	Living	Dead
0.000,000,01	Living	Dead	Living	Dead	Living	Sick
0.000,000,001	Living	Living	Living	Living	Living	Dead
Protection obtained against	1,000,000 doses		10 doses		10,000 doses	

decided to determine which specific ingredient of the Huntoon antibody solution was effective. We may say at the outset that the particular lot of the Huntoon solution tried out against the fixed Types I, II and III as a preliminary test, showed its best protection against Type I pneumococcus, less protection against Type II and no protection against Type III (table 1). These tests as well as those subsequently to be described were carried out in duplicate. It seemed quite clear, therefore, that the Type III antibodies were not responsible for the cross protection which the Huntoon solution seemingly afforded against the "three strains."

We desired at this point to investigate the protective specificity of monovalent anti-pneumococcus horse sera of the three fixed types. Unfortunately we were able to obtain only Type I horse serum where the history of the horse made it certain that it had never received injections with others than Type I pneumococcus. This serum tried out against the three fixed Types I, II and III showed definite but slight cross protection which was confirmed when repeated (table 2).

In order to investigate the cross protective power of Type II and III sera as well as to rule out all doubts as to the specificity of the antigen used to obtain these sera, we determined to prepare our own monovalent protective sera in rabbits.

As is seen in table 3, the sera obtained from rabbits also showed slight but distinct cross protection, and this despite the fact that they showed no cross agglutination with the other fixed type strains. It is to be observed that the cross protection obtained showed some reciprocal relation between Types I and III. Another interesting point brought out in this table, is that the cross protective potency varied with the different rabbits. This is all the more interesting when we consider that each rabbit was injected with washed and killed organisms obtained from the same beef infusion broth culture. Moreover, numerous fishings from the cultures failed to show by agglutination any admixture of the strains. We may say too, that on the whole the protective specificity of rabbit sera seemed to us to be greater than that of the horse sera.

TABLE 3

CROSS PROTECTION BY RABBIT MONOVALENT SERA

The figures indicate the number of fatal doses against which protection was obtained

	SERUM NUMBER	TYPE I PNEUMOCOCCUS	TYPE II PNEUMOCOCCUS	TYPE III PNEUMOCOCCUS
Type I sera.......	26	1,000,000,000	10,000	0
	28	10,000,000	0 to 10	1,000
Type II sera......	29	0	100.000,000	*
	30	0	10,000,000	100
	31	0 to 10	10,000,000	0 to 10
	35	0	1,000,000,000	0 to 10
Type III sera......	33	10,000	0	1,000,000
	38	100	0 to 10	100,000

*Not done, short of serum.

Finally, rabbit protective sera were tried out against the "three group IV strains." The results of these tests are shown in table 4. It is seen that of the three strains, two could not be protected against by these sera. In strain

11, however, cross protection was afforded. Marked protection was obtained from Type II serum and slight protection from Types I and III sera. Of the Type III sera, one afforded some protection, the other showed none.

TABLE 4

PROTECTION AFFORDED BY RABBIT MONOVALENT PROTECTIVE SERA AND FELTON'S SERUM AGAINST THE "THREE STRAINS"

The figures indicate the number of fatal doses against which protection was obtained

GROUP IV STRAINS	HUNTOON'S SOLUTION	TYPE I SERA		TYPE II SERA	TYPE III SERA		FELTON SERUM
		No. 25	No. 28	No. 31	No. 33	No. 52	
No. 9	0 to 10	0	0	0 to 10	0	0	0
No. 11	0 to 10	1,000	1,000	1,000,000	1,000	0	10,000
No. 22	0 to 10	0	0	0	0 to 10	0	0 to 10

TABLE 5

PROTECTION TESTS AGAINST CULTURES OF FIXED TYPES I, II AND III PNEUMOCOCCI WITH FELTON'S REFINED SERUM

DOSES OF CULTURES IN FRACTIONS OF 1 CC.	TYPE I PNEUMOCOCCUS		TYPE II PNEUMOCOCCUS		TYPE III PNEUMOCOCCUS	
	Felton's serum	Virulence control	Felton's serum	Virulence control	Felton's serum	Virulence control
1.0	Dead				Dead	
0.5	Living				Dead	
0.1	Living				Dead	
0.01	Living				Dead	
0.001	Living				Dead	
0.000,1	Living				Living	
0.000.01	Living				Living	
0.000.00.1	Living		Dead	Dead	Living	
0.000,000,1	Living	Dead	Dead	Dead	Living	Dead
0.000,000,01	Living	Dead	Living	Living	Living	Dead
0.000,000,001	Living	Living	Living	Living	Living	Living
Protection obtained against..	100,000,000 doses		No protection		10,000 doses	

A preparation of Felton's refined anti-pneumococcus serum which is prepared from Type I horse serum, was tested for its cross protective power against the three fixed Types I, II, and III pneumococci. The results are seen in table 5. This serum, as is seen in table 4, also afforded some protection against strain 11 but none against strains 9 and 22.

DISCUSSION

The chief point of interest which arises from this work is the cross protection between types which is shown by horse as well as rabbit sera. In the latter, cross protection is found only in Types I and III sera. An interesting point, which was continually brought to our attention, was the more or less constant reciprocal relation between Types I and III. It appears from our tables that in so far as the production of protective antibodies is concerned, Types I and III pneumococci bear a stronger antigenic relation to one another than do either of these with Type II or the group IV strains tested by us.

The next point brought out, which though perhaps of less importance, was nevertheless the original question which we desired to answer, is that none of the 37 group IV strains which we studied, could be protected against by the use of Huntoon's polyvalent antibody solution. This sample of the solution moreover, contains no demonstrable protective antibodies against Type III pneumococcus.

The fact that the several monovalent rabbit sera afforded some protection to one of the "three group IV strains" despite the inability of Huntoon's solution to afford protection, suggests that the considerably greater potency of the rabbit sera was responsible for this. Nevertheless, no protection was afforded by these rabbit sera to either of the remaining two of the "three strains." This suggests that only occasional members of group IV are amenable to protection by the several monovalent sera. It is quite conceivable that had we carried out protection experiments with the rabbit monovalent sera against more of the group IV strains, some might have been found to be amenable to protection by large amounts of antibody.

The classification of sera by their agglutinin content, while very valuable, gives no accurate indication of their protective titer and certainly no idea of their cross protective power. We have tried to point out the importance of the latter and we hope that this short contribution may stimulate interest in this direction.

Further observations on the cross protective power of sera should be of great practical importance.

CONCLUSIONS

1. A sample of Type I monovalent anti-pneumococcus horse serum examined by us showed definite cross protection against the Type III strain.
2. Monovalent anti-pneumococcus rabbit sera also showed cross protection. The protective power against the homologus strains was on the whole greater than that of horse sera.
3. The cross protective titer of these sera varied with the rabbits from which they were obtained.
4. Cross protection while clearly present between Types I and III sera, was far below the protection titer against the homologous strain. It is probably too slight to have therapeutic effect.
5. Despite this cross protection, all the rabbit sera studied by us were specific in their agglutinin content.
6. Protection tests with rabbit monovalent sera were made against three group IV strains. In one of these strains protection was present to the extent of from 1000 (Types I and III) to 1,000,000 fatal doses (Type II serum), depending on type of serum employed and to a lesser extent upon the rabbit, from which the serum was obtained.
7. The importance of determining the cross protection power of monovalent sera has been discussed.

It is a real pleasure to record our indebtedness to Dr. William H. Park, Director of these laboratories, for his continued help and interest in this investigation.

For bibliography see the original article.

THE DISTRIBUTION OF THE IMMUNE BODIES OCCURRING IN TYPES I, II AND III ANTIPNEUMOCOCCUS SERUM

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Edwin J. Banzhaf

Avery states that the immune bodies occurring in Types I and II are completely precipitated by 38 to 42 per cent. saturation with ammonium sulphate, and that they were incompletely precipitated by (a) ammonium sulphate in less than 38 per cent. saturation, (b) saturation with sodium chloride, (c) dilution and saturation with carbon dioxide and (d) removal of crystalloids by dialysis. He states that the most practical purification appears to be precipitation by 38 to 42 per cent. saturation with ammonium sulphate. The higher saturation, *i.e.,* the 42 per cent., corresponds to about 47.6 cc. of saturated ammonium sulphate solution.

Felton finds that a 10 times dilution with distilled water containing 4 per cent. N/1 phosphoric acid per volume of antiserum will, with Type I ,completely precipitate the immune bodies. He has been less successful with some of the antisera of Types II and III, especially those of low protective value. We have corroborated Felton's findings with Type I. Practically a complete precipitation of the immune bodies occurs when diluted with distilled water containing 4 per cent. N/1 phosphoric acid per volume of antiserum. Felton's same technique on Types II and III has given us varying results.

Using ammonium sulphate as a precipitating agent, we corroborated Avery's work in finding that the immune bodies were completely precipitated by half saturation with saturated ammonium sulphate solution. We also found that the methods of separation of the so-called euglobulin and pseudoglobulin by ammonium sulphate and sodium chloride showed immune bodies in both glubulins. We found, however, that that portion of so-called euglobulin which is precipitated with 30 per cent. saturated ammonium sulphate solution, saturated sodium chloride or 12½ per cent. dried sodium sulphate, contained after dialysis only 8 to 10 per cent. of the total immune bodies. This holds true for all three types.

Using Felton's technique for diluting these dializates, the white precipitate that is obtained contains practically all the immune bodies from these first fraction precipitates or so-called euglobulins.

The filtrate containing the globulins soluble in 30 per cent. saturated ammonium sulphate solution, and precipitated by adding saturated ammonium sulphate solution to half saturation contains after dialysis about 90 per cent. of the immune bodies.

The saturated sodium chloride soluble globulins, precipitated by the addition of only 25 per cent. saturated ammonium sulphate solution, contains after dialysis about 90 per cent. of the immune bodies. The filtrate containing the globulins soluble in 12½ per cent. dried sodium sulphate, and precipitated by adding dried sodium sulphate up to 18½ per cent. at 36°C., also contains, after dailysis, about 90 per cent. of the immune bodies.

Using Felton's technique for diluting these dializates, the white precipitate that is obtained contains practically all the immune bodies from these second fraction precipitates. This holds true for all three types. In all instances where Felton's white precipitate is obtained in the three types of pneumococcus antisera, the immune bodies are found in his white precipitate.

The albumins have no immune bodies, and no white precipitate appears when diluted with distilled water. Heating the antisera to 58°C. for two hours destroys about 60 per cent. of the immune bodies in all three types.

Heating to 56°C. for one hour does not impair its protection value.

All protection tests were made by Miss W. Carey Noble, to whom I am greatly indebted.

For bibliography see the original article.

PRODUCTION OF STAPHYLOCOCCUS ANTITOXIN IN HORSES[1]

Published in The Journal of Immunology, 1927, Vol. XIII, pp. 25-29

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It has been shown by one of us that most strains of *Staphylococcus aureus* from pathogenic sources produce a poison which in 0.1 cc. amounts causes marked necrosis when injected into the skin of rabbits. At the time of writing these papers we were not aware that von Lingelsheim as early as 1900 described experiments in which he inoculated subcutaneously 8 to 10 cc. of sterile filtrates of broth cultures of *Staphylococcus aureus* and obtained marked inflammatory changes and sometimes necrosis. More recently, Neisser and Wechsberg confirmed these findings with smaller injections (0.2 to 0.3 cc.) of toxic filtrates. These observers found as we did, that this necrotizing poison was destroyed when heated to 58° for one hour.

On further analysis we showed this poison to be an exotoxin, as the serum of rabbits immunized thereto neutralized it in multiple quantities. By the method of titration and calculation used, after 3 to 7 intracutaneous injections of increasing doses of toxin, rabbit serum contained 1250 to 5000 units of antitoxin.

With a view to the possible use of staphylococcus antitoxin in human infections of staphylococcus origin, we undertook the immunization of horses. This was thought advisable in order to get greater quantities of serum than could be obtained from rabbits, and because it was hoped that horses, as it has been shown in the production of the other antitoxins, would also prove to be a better staphylococcus antitoxin producer than the rabbit.

In some of the horses the first few injections were made by the intracutaneous route. After such injections there was usually considerable swelling at the site of injection and occasionally the formation of a scab, 2 by 3 cm. in diameter. The other injections were given subcutaneously. There was no apparent local reaction after the subcutaneous injection, but in those horses which were getting large injections of toxin at close intervals (see table) there was frequently a rise of temperature of 101° to 104°F.

Table 1 gives some of the data on the immunization of 7 horses with the staphylococcus toxin. It is seen that 5 of the 7 horses died during the course of immunization after six weeks to three months' treatment. These horses died with symptoms of weakness and prostration, and at autopsy showed nothing abnormal macroscopically. It is noted in table 1 that these horses had been given injections of toxin at short intervals, in most instances one to two days apart. Furthermore—not indicated in table 1—the injection doses were increased rapidly, so that after three weeks treatment they were getting 50 to 75 cc. of toxin. We believe that this strenuous treatment may account for the deaths, since the last 2 horses immunized (nos. 6 and 7), in

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[1] We are indebted to Dr. E. L. Cornman, Marietta, Pa. for the immunization of some of the horses and to Miss Anne Gunthur for the preparation of some of the toxin.

TABLE 1

HORSE NUMBER	PERIOD OF INJECTION	NUMBER OF INJECTIONS	AMOUNT OF FIRST INJECTION	INTERVALS BETWEEN INJECTIONS	TOTAL AMOUNT TOXIN INJECTED	LARGEST AMOUNT TOXIN INJECTED	DATE OF DEATH	APPROXIMATE NUMBER OF ANTITOXIN UNITS IN 1 CC. OF SERUM	DATES OF BLEEDING
			cc.		cc.	cc.			
1	March 2, 1925, through May 20, 1925	17	0.5	First 10 injections 3 or 4 days	1051	300	May 23, 1925	30,000	May 6, 1925
				Last 7 injections 7 to 10 days				30,000	May 19, 1925
2	May 18, 1925, through July 14, 1925	23	10	First 12 injections daily	1205	85	July 28, 1925	7,000	July 22, 1925
				Last 11 injections 4 to 10 days					
3	August 20, 1925, through September 28, 1925	15	10	1 to 5 days	670	85	October 5, 1925		
4	November 20, 1925, through December 31, 1925	15	10	1 to 5 days	610	60	January 2, 1926	7,000	December 28, 1925
5	January 9, 1926, through March 20, 1926	2	10	2 to 4 days except in 2. instances	995	100	March 31, 1926	15,000	February 26, 1926
				7 and 11 days				20,000	March 19, 1926
6	March 1, 1926	20	1	First 10 injections 3 and 4 days	2401	400		20,000	May 21, 1926
				Other injections 7 to 21 days				30,000 20,000	June 11, 1926 July 1, 1926
7	March 1, 1926	20	1	First 10 injections 3 and 4 days	2401	400		30,000 10,000	July 12, 1926 May 21, 1926
				Other injectons 7 to 21 days				10,000 10,000 25,000	June 11, 1926 July 1, 1926 July 12, 1926

which injections larger than 12 cc. were given at eight- to ten-day intervals, are in good condition at the present writing after five months treatment.

The amount of antitoxin produced by the horses is given in table 1 (column 10). It is seen that even in the horses which withstood the immunization for a short period, the amount of antitoxin produced was greater than that contained in our strongest rabbit serums and in several horses many times stronger.

In contrast with normal rabbit serum which seldom contains even slight amounts of staphylococcus antitoxin, normal horse serum usually contains a definite quantity of antitoxin. Of 5 normal horse serums titrated for staphylococcus antitoxin, 1 contained no antitoxin, 3 contained 50 units and one 100. A diphtheria antitoxin titrated contained 200 units.

The titration for antitoxin was carried out as in our previous work. There are, however, a few points in carrying out the titrations which should be emphasized. They are as follows:

1. Only healthy rabbits should be used, as an animal not in good condition for any reason may not react even to a strong toxin.
2. A strong toxin, viz., one which produces a + reaction diluted 1:40 should always be used in any titration.
3. In every rabbit used for titrations of an antitoxin, controls of a mixture of antitoxin of known strength and the toxin, and toxin alone with equal amount of salt solution should be injected. In accurate determinations an added control of toxin diluted to the point where it will produce a plus reaction should be inoculated.
4. The antitoxin and toxin controls should always be the last injections made on the rabbit. This last point is important because if considerable excess of antitoxin over that necessary to neutralize the toxin is used in one or more mixtures injected in the rabbit, this excess of antitoxin in itself may wholly or partially neutralize the later inoculations and the titration must be rejected and set up again on another rabbit with higher dilutions of antitoxin.

For bibliography see the original article.

MENINGEAL CONDITIONS NOTED DURING THE EPIDEMIC OF INFLUENZA

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Josephine B. Neal

In reviewing the cases of meningeal involvement developing during the epidemic of influenza, three points attract attention:

1. Very few cases of meningitis have been due to the influenza bacillus. We have seen but two, and have examined the fluids of a third case. In previous years we have seen as many in an equal length of time. If this epidemic were due to the influenza bacillus, it seems strange that the organism did not more frequently invade the meninges.

2. During October and the early part of November, we saw a larger number of cases of epidemic meningitis than is usual at that season, and the number dropped off subsequently. The great majority of these cases gave a history of an attack, clinically influenza, immediately preceding the development of the meningitis. In some instances, the meningitis appeared as the influenza was subsiding, in other instances after convalescence was established. The cases themselves were not unusual in any way. Some were mild and some severe—about as the cases usually run. Cultures of the spinal fluids were made on blood agar as well as on glucose ascitic agar to see whether hemoglobinophilic bacilli were present with the meningococci, but there was no evidence of a mixed infection. Whether the resistance of the individual was lowered locally in the mucous membrane of the nasopharynx and generally by the attack of influenza, thus making him fall more readily a victim to meningitis, or whether the meningococcus attained a heightened virulence through its association with the causative agent of the influenza epidemic, is a question entirely open to discussion.

3. In about twenty cases we have seen patients, usually during convalescence from attacks clinically influenza, in which meningeal symptoms have developed. There were present headache, some stiffness of the neck, Kernig's sign, and extreme apathy and drowsiness—in many ways the picture of an early tuberculous meningitis. Lumbar puncture has revealed a clear fluid under increased pressure. This fluid has shown an increase from slight to marked, in the number of cells, with a preponderance of mononuclears, a well marked increase in the albumin and globulin, a normal reduction of Fehling's solution, and no organisms either by smear or culture. In most instances a Wasserman test has been performed, and in all cases have been negative. Guinea-pigs inoculated with a number of fluids suffered no ill effects. Such a spinal fluid in no way suggests the invasion of the meninges by a pyogenic micro-organism. The mononuclear cells and the normal reduction of Fehling's solution are practically never present in a pyogenic meningitis. This in addition to the absence of organisms by smear and culture is evidence against a meningitis.

This spinal fluid reaction is quite different from that usually found in meningism occurring in pneumonia, gastro-intestinal conditions in children and acute infections. In these conditions, while the meningeal symptoms are often more pronounced than in the cases above described, the spinal fluid, with rare exceptions, is normal in the chemical and cytologic constituents. The exceptions referred to occur mainly after severe and prolonged convulsions, in the convulsive type of whooping cough and in cases in which the puncture has been done shortly before death, the circulation having been depressed for some time. In these instances, the changes in the cytology and chemistry are, as a rule, less than have been found in the influenzal cases. The spinal fluid changes found in the cases described are similar to those in cases of poliomyelitis and poliencephalitis. From the spinal fluid examinations and the clinical picture it seems possible that we are dealing with an encephalitis secondary to the influenzal attack. With one or two exceptions, the patients have improved after the lumbar puncture and made a good recovery.

In the original article several case histories are given.

INFLUENZAL MENINGITIS

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Josephine B. Neal

Meningitis due to the Pfeiffer bacillus ranks fourth in our experience among the types of purulent meningitis.

Table 1 shows the incidence of the different forms of purulent meningitis among the cases of meningeal involvement that have been studied by the Meningitis Division during the past 10 years.

TABLE 1

CASES OF PURULENT MENINGITIS

Epidemic Cerebrospinal Meningitis	541
Pneumonic Meningitis	61
Streptococcic Meningitis	49
Influenzal Meningitis	32
Staphylococcic Meningitis	9
Total	692

The cases of influenzal meningitis that have been reported and collected by various workers are given in a table in the original paper.

The cases reported by Simon, Wollstein, Torrey and Stone represent reports collected from the literature in addition to their own cases. So far as I have been able to determine there are no duplications. The cases credited to Koplik, Dunn, and the Meningitis Division were seen by the workers in question.

Dr. Wollstein made a careful study of the published reports and from these, in addition to her own investigations, concluded that in majority of fatal cases a general blood infection occurs. She also pointed out that the development of influenzal meningitis is frequently preceded by otitis, rhinitis, clinical "influenza," or other involvement of the respiratory tract.

In our series of cases the diagnosis was made by finding in the spinal fluid the gram negative hemoglobinophilic bacillus described by Pfeiffer. In smears from the spinal fluid these bacilli show a high degree of pleomorphism, a fact observed by several other workers. An interesting point was brought out by Dr. Povitzky, of the Research Laboratory who found that 4 out of 7 strains from cases of influenzal meningitis belonged by agglutination to the same type. On the other hand, the strains isolated from the respiratory tract during the epidemic of influenza, so-called, showed only a slight tendency to fall into groups when studied by Miss Valentine and Miss Cooper.

It is, of course, only by the bacteriological study of the spinal fluid that the diagnosis of influenzal meningitis can be made during life, as there is nothing in the history, the clinical picture of the case, or the other characteristics of the spinal fluid to distinguish it from other forms of purulent meningitis. Indeed, from a clinical standpoint, the disease is especially liable to escape diagnosis, since so large a proportion of the cases are found in infants in whom the diagnosis of meningitis is generally most difficult. It is evidently a disease of very young children. In our series of 32 cases, only 2 patients were more than 5 years old, one of these being 7 and one 18 years of age. Sixteen of our cases were in children one year old or less.

We were able to locate a probable primary focus in only 8 instances and, of these, 6 were cases of pneumonia, one clinical "influenza" and one a cold with cough. I am, however, unable to say whether or not these primary infections were due to the influenzal bacillus. It is certain that meningitis due to the meningococcus does not develope frequently during or immediately following pneumonia.

Lumbar puncture is all too often delayed because the physician feels he is dealing with a meningism or with a meningitis secondary to the pneumonia probably due to the pneumococcus or streptococcus.

As to the seasonal distribution of cases, a tendency is shown for the disease to develop in the fall and early winter. But it is particularly interesting to note that in the fall of 1918, when the epidemic of influenza was raging in New York City, we saw only one case of meningitis due to the Pfeiffer bacillus.

This has seemed to us good evidence against the Pfeiffer bacillus being the causative factor of this epidemic. If the organism had been present in such vast numbers and with such heightened virulence as to have caused such an epidemic, it seems impossible that there should not have been an increase in the number of cases of influenzal meningitis. This has been commented upon in an earlier paper, and Stone also called attention to the same point in his experience in Texas.

As stated in a previous paragraph, there is nothing characteristic in the symptomatology of influenzal meningitis. Two patients had even the hemorrhagic rash, which is so often considered diagnostic of epidemic meningitis. The course of the disease varies greatly, from two or three days to a month or more but is usually well over a week. A table showing in tabular form, certain points of interest in the cases we have seen is given in the original article.

A study of the 13 cases in which recovery took place, shows that the treatment was as follows: In nine instances lumbar punctures alone was used; in one instance electrargol was given intravenously daily for a week; in two instances anti-influenzal serum was used; in one, vaccine was given intraspinally.

An anti-influenzal serum prepared by Wollstein in 1911, produced favorable results in experimental influenzal meningitis in monkeys. This was the serum used in pateints who recovered as reported by Torrey. The preparation has not had a fair trial clinically as the disease is too rare to furnish an adequate number of cases and the serum is often administered late. Dunn reports that he has used it in 11 cases without a recovery, but that too few doses were given to furnish an adequate estimate of its value. We have used it in five cases. In three of these only one or two injections were given, in the other two the injections were repeated about ten times in one instance and fifteen times in the other. These last two patients showed temporary improvement clinically; the organisms decreased greatly in number and became largely intracellular; death, however, finally resulted. The serum certainly should be used when available.

A brief description of one case that recovered may be of interest. No. 28 in our series.

Phyllis McA., 2½ years of age, had been well except for a mild cold and cough four to six weeks earlier. She had not recently been in contact, as far as we know, with any sick person. She became ill, suddenly, August 21st, 1920, with headache, vomiting and fever ranging from 99° to 102°. Two lumbar punctures were done, August 22nd and 26th. The spinal fluids were sent to us for examination and the second one showed the Pfeiffer bacillus. On August 28th the child was seen by Dr. Jackson of the Meningitis Division. Twenty-five cubic centimeters of turbid fluid was withdrawn and 500 million of a stock influenza vaccine was injected intraspinally.

The child's mother objected to lumbar punctures especially in view of the fact that we could hold out little hope for recovery, and it was not possible to persuade her to permit regular treatment.

On August 31st another puncture was made, 1000 million of vaccine being given mixed with antimeningitis serum, as the smear from the previous fluid had showed coccoid forms which suggested that the meningococcus might also be present.

Further study showed that this fluid as well as the previous ones contained the Pfeiffer bacillus in pure culture. At this point the case showed some improvement, and no further treatment was permitted until September 9th, when the child became suddenly worse with a rise of temperature and vomiting. A puncture was made and influenza vaccine was again administered. This was repeated about every other day, the case showing considerable improvement. An autogenous vaccine was used as soon as it could be prepared. On September 18th the fluid for the first time gave a negative culture, the previous eight fluids having been positive. At this puncture in addition to the vaccine, anti-influenzal serum, (prepared by the Research Laboratory, but not available until this time) was given. Therefore, in this particular case at least, the serum cannot be credited with the disappearance of the organism and the ultimate recovery of the case.

The administration of combined serum and vaccine was repeated on the 20th and 22nd of September. On September 24th and October 2nd, lumbar puncture was done for the relief of pressure, no injection being made. The child showed steady improvement in general condition. Deafness which had developed early in the illness and had seemed to clear up, returned about the middle of September. It did not seem to be complete and was apparently showing a little improvement when the child went to the country the middle of October. Her general condition at that time was excellent.

In several cases of meningitis not due to the meningococcus, we have used a vaccine intraspinally with the hope that its gradual absorption into the blood stream would bring about whatever beneficial effects result from vaccine given intravenously without the degree of shock usually following that method of administration. We had the opportunity of making several injections in this way in two cases each of streptococcic and staphylococcic meningitis, with recovery in one case of staphylococcic meningitis and temporary improvement in the three other cases. The reaction following this treatment has never been very severe, and we have seen no harmful results, though in some cases we have worked up to as large a dose as five billion, diluted in about 10 cc. of normal saline. The vaccines used were either stock vaccines of the same variety as the organisms present, or autogenous vaccines when such could be prepared, as it was desired to get any specific effects that might be obtained in addition to the general protein reaction. Sampietro and others have recently recommended homologous vaccine in protein-shock treatment as it seems to give better results and to be less toxic.

The intravenous administration of vaccine has been used also in treating meningitis. Typhoid vaccine was used in this way in a case of epidemic meningitis on the Fourth Medical Division at Bellevue Hospital. This patient had developed a walling off of the spinal fluid, repeated lumbar punctures resulting in dry taps. The condition was getting worse and indeed seemed to be quite desperate. The intravenous administration of the vaccine was followed by considerable shock. The man improved temporarily but died about four months later.

A case of blocked epidemic meningitis in a baby, treated with intraventricular administration of serum followed by vaccine intravenously and recovery, has recently been reported by de Angelis.

Recoveries from purulent meningitis, due to organisms other than the meningococcus, are few. Most of these have occurred after lumbar puncture alone. In view of this fact, it cannot be asserted that intrsapinal vaccine treatment cured the case of staphylococcus and of influenzal meningitis above described nor that the intravenous administration of vaccine cured the case of blocked epidemic meningitis to which we referred. However, recovery did follow the use of vaccine in these cases.

In the absence of methods promising better results it seems worth while in addition to the use of a homologous serum where such is available, to try the intraspinal or intravenous administration of vaccine in suitable cases. This course would be indicated under the following conditions: in cases of meningitis not due to the meningococcus, in blocked forms of epidemic meningitis and in other cases of epidemic meningitis which do not yield readily to serum treatment.

For bibliography see the original article.

EXPERIENCE WITH MORE THAN ONE HUNDRED CASES OF EPIDEMIC ENCEPHALITIS IN CHILDREN

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Josephine B. Neal

The scope of this paper permits only the briefest reference to the extensive literature that has grown up during the last four years around epidemic encephalitis. The articles referring especially to the disease in children, however, have been comparatively few. Batten and Still, Netter, Comby, Heiman, and others have contributed to this phase of the subject, reporting, however, comparatively few cases. Indeed, earlier, it was thought that adults were much more subject to the disease than children. In fifty-four early cases studied by Netter, 77 per cent. of the patients were more than 15 years of age, and in London 86 per cent. of patients in early cases were more than 10 years of age. In an earlier article from the meningitis division, it was pointed out that perhaps a number of cases in children were overlooked. as approximately half our patients were 15 years of age or younger. Further epidemiologic study has confirmed this opinion. In a recent and very thorough study by Happ and Mason slightly more than half their eighty-one cases were in this age category. Up to the middle of May 1921, we had studied 274 cases in exactly half of which (137) the patients were 15 years of age or younger. The exact age distribution is shown in the accompanying table. One hundred and twelve, or 40 per cent., of the patients were 10 years of age or younger.

In contrast to this, in about 65 per cent. of cases of epidemic meningitis and about 90 per cent. of cases of poliomyelitis, the patients are under 10 years of age. As regards sex, about 50 per cent. more males than females are attacked by the disease, but this also holds true of epidemic meningitis and poliomyelitis. The seasonal distribution of our cases in New York City is indicated in the chart, and shows that the maximum

AGE DISTRIBUTION

Age	No of Cases
3 months	4
From 3 to 6 months	4
From 6 to 12 months	8
From 1 to 2 years	20
From 2 to 5 years	29
From 5 to 10 years	47
From 10 to 15 years	25
Total	137
From 15 to 20 years	15
From 20 to 30 years	56
From 30 to 40 years	33
From 40 to 50 years	24
Over 50 years	9
Total	274

number of cases occurs in the first quarter of the year. Our sporadic cases of epidemic meningitis during the last eleven years have shown the maximum number in April, May and June, while poliomyelitis is usually at its height during the summer months. While epidemic encephalitis is accepted as being an infectious disease, the percentage of those susceptible to it is certainly very low. In only one instance have we seen two cases in a family. The two patients became sick on the same day, and no outside source could be traced. The reports of instances of traceable contagion are very few. This difficulty in tracing infection is encountered also in epidemic meningitis and poliomyelitis, and may point to a high resistance to these diseases on the part of the majority of people. We have observed two or three points in connection with the study of our cases which may be of some interest. One patient while convalescing from encephalitis contracted epidemic meningitis, probably from a patient in an adjoining bed in the hospital. Instances of such direct contagion in hospitals, as we all know, are very rare. One patient gave a history of epidemic meningitis a year before. Two patients were mentally defective. Four had suffered from attacks that were probably poliomyelitis. One patient after a year of good health died from a second attack of encephalitis, so far as the diagnosis could be made in both instances.

No attempt has been made to divide our cases into clinical types because we are evidently dealing with a virus that may attack any part of the central nervous system or any combination of parts. This obviously gives rise to a great variety of types. Moreover, a given case in its development often passes through several phases representing different types. For these reasons, an attempt at classification has seemed undesirable.

SYMPTOMATOLOGY

The symptoms in children do not differ greatly from those in adults. A rather larger percentage of children show a sudden onset, and the course of the disease is ordinarily shorter than in adults, although the mortality is approximately the same. The symptoms comprise both those of a generalized infection, such as fever, headache, vomiting, constipation and malaise, and those more directly referable to the central nervous system. The latter show an almost endless variety. Lethargy was so prominent a symptom in many of the cases that the term "lethargic" was earlier used in describing the disease. This, however, is by no means a constant symptom. Indeed, insomnia is an equally striking phenomenon in many cases, and in some patients both conditions may be present at different times. Convulsions are common, especially in younger children. In many cases delirium is present, occurring either for a considerable time or for short intervals, lasting not more than ten or fifteen minutes and being repeated frequently.

Ocular disturbances are of very frequent occurrence and are of wide variety. They may take the form of diplopia, blurring of vision, blindness, ptosis, either unilateral or bilateral, strabismus, nystagmus, and occasionally peculiar motions of the eyes. In one instance there occasionally took place convulsive twitching of the eyes which then snapped quickly to the left, and remained fixed for two or three minutes. These seizures occurred at frequent intervals during the height of the disease, but disappeared entirely as the patient recovered. In another case the eyeballs performed in succession a curious, nearly complete rotation with the pupils looking inward. While one eye was in motion the other eye remained quiet, but there was more or less twitching of the lips and of the face muscles on the same side.

Hippus has been observed in two or three cases in adults. I have not seen it in children.

Paralyses of wide distribution may occur. The cranial nerves are more often affected. Facial paralysis or paresis, either unilateral or bilateral, is often present and probably causes the masklike expression so often noted. In some instances the neck muscles have been affected; in one case this was unilateral. Inability or unwillingness to swallow may occur to such an extent as to make tube-feeding necessary. Disturbances of speech are not uncommon. The one I have observed most frequently is that in which the patient speaks in a slow, monotonous slurring voice, with considerable delay before answering questions. Some cases are extremely talkative. In a number of instances under my observation, patients have refused to speak for a varying length of time. Twitchings, either general or localized, are a very common phenomenon. They vary from a fine tremor to choreiform movements. Catatonia is not infrequent. The reflexes show all varieties of change. They may be increased, decreased, lost, equal or unequal. The Babinski sign and ankle clonus are not uncommon. Retention of incontinence of urine occasionally occurs. Constipation is often obstinate. Profuse sweating is fairly common. The more typical meningeal symptoms, such as stiffness of the neck and the Kernig sign, are not common, unless associated with some degree of general spasticity.

The course of the disease varies greatly, from two or three weeks to many months. The prolonged cases, lasting several months, are less common in children than in adults. The course of the disease is often marked by sudden rather transitory changes either for the better or the worse, and one must, therefore, be guarded in making a prognosis from a single inspection of the patient. A sharp and unexplained rise in temperature occasionally occurs. Pneumonia may develop during the course of the disease and is a dangerous complication. A rather common type of the disease in children is that in which there are none of these more striking phenomena. There is a history of a gradual onset, a varying degree of drowsiness, headache, vomiting, constipation, low, irregular temperature, and some changes in the reflexes. The diagnosis of early tuberculous meningitis is suspected, but after one or two lumbar punctures, in which the spinal fluid does not corroborate this diagnosis, the case clears up.

Laboratory Findings

The blood count in epidemic encephalitis varies from normal to one showing a moderate degree of leukocytosis, perhaps up to 15,000 or 20,000. Blood cultures are sterile. The urine is negative or shows a mild degree of nephritis common in acute infectious conditions. The examination of the spinal fluid throws more light on the subject than any other laboratory procedure. The spinal fluid shows practically the same picture as in poliomyelitis. It is clear and usually increased in amount. A web rarely forms. The cell count may sometimes be normal. It is usually moderately increased, ordinarily to no more than 100 or 150, though in one case it ran as high as 1500. The mononuclears usually predominate, but as in poliomyelitis there may occasionally occur a preponderance of polymorphonuclears. As in poliomyelitis, the fluid may in rare instances be slightly blood tinged, probably indicating a more than usually severe hemorrhagic process. The albumin and globulin are increased, usually slightly to moderately. The glucose, as measured by the qualitative reduction of Fehling's solution, is normal. Quantitative studies by various workers, among them Foster, have shown that it is sometimes slightly increased above normal. I do not feel that this knowledge is of great diagnostic value. Different workers vary in their estimation of the glucose in normal fluid. In quantitative work done in the meningitis division in 1916, is was found by Kahn that the glucose in poliomyelitis fluids frequently ran from 0.08 to 0.10 per cent., also an increase above normal.

The Wassermann reaction is negative. The colloidal gold curve is not characteristic. When the disease first made its appearance, many of the English writers and some in France and America reported normal spinal fluid findings, but most of the recent writers have reported findings similar to those described above. It will be seen from this description that these findings are not diagnostic of epidemic encephalitis, as similar findings may occur in poliomyelitis, in brain abscess, brain tumor, and except for the Wassermann test and colloidal gold test, in syphilitic conditions of the central nervous system, all being conditions in which the brain substance is primarily involved, with the meninges being secondarily affected. Such findings also occur early in tuberculous meningitis. They do show, however, that there is an inflammatory reaction in the central nervous system, and they are of value in ruling out other conditions in which meningism occurs with symptoms referable to the central nervous system but without an actual inflammation being present.

While much work has been done to determine the specific etiologic factor of epidemic encephalitis, the results obtained by different workers have been by no means in agreement. The work of Loewe, Strauss and Hirshfeld, has been in part duplicated by Levaditi and Harvier. Other workers have reported negative results in their attempts to corroborate this work. In view of these facts we must feel that the exact etiologic factor has not been fully established. Loewe, Strauss and Hirshfeld, and Levaditi and Harvier believe that they have established that the disease can be transmitted to rabbits and monkeys, and that it is due to a filterable virus in many respects similar to that considered to be the cause of poliomyelitis.

Differential Diagnosis

Since this disease is so protean in its clinical manifestations and since there are no laboratory findings that are absolutely specific, the diagnosis must be made largely by exclusion. In the case of children, tuberculous meningitis is the disease from which it must be most often differentiated, and this differentiation is by no means easy. The onset, while usually gradual in both, is more often abrupt in encephalitis, and in this type of case death may occur within seven to ten days, a shorter duration than tuberculous meningitis usually has. On the other hand, patients suffering from encephalitis sometimes die after a course of two or three months, a longer period than cases of tuberculous meningitis show. Diplopia is occasionally present in tuberculous meningitis, and transient paralyses especially of the cranial nerves are common. So also is loss of reflexes. Other neurologic manifestations that have been described in epidemic encephalitis are rare. Evidences of tuberculous lesions elsewhere, positive tuberculin reaction, tubercles in the choroid and so forth, are also of help in making the diagnosis of tuberculous meningitis. The examination of the spinal fluid in the last analysis must often be the final factor. While the spinal fluids may not be dissimilar early, as a rule even fairly early the spinal fluid in tuberculous meningitis will have a higher cell count and a greater increase in albumin and globulin than is the rule in encephalitis, even though the reduction of Fehling's solution be normal. As the disease progresses, the reduction of Fehling's solution in cases of tuberculous meningitis will practically always become diminished or absent, and it is possible in a large percentage of cases to demonstrate the tubercle bacilli in the smear. If this cannot be done, the result of animal inoculation must be awaited. Films form almost invariably in fluids from tuberculous meningitis, and only occasionally in fluids from epidemic encephalitis.

The diagnosis from poliomyelitis, especially the encephalitic form, is practically impossible except by neutralization tests of the blood serum. These tests are difficult to perform and are not always conclusive. Amoss has shown that the serum from recovering cases of encephalitis has no power in neutralizing poliomyelitis virus. Of course, the onset of poliomyelitis, even of the encephalitic type, is more frequently abrupt than is that of encephalitis. There is likely to be a higher temperature early, which soon subsides; there is more frequently diarrhea in poliomyelitis, while constipation is the rule in encephalitis. The pupils in poliomyelitis practically always react to light, while in encephalitis the reaction is often sluggish. Moreover, even in epidemics of poliomyelitis, the encephalitic form is rare. The age and season must also be considered. All these factors are helpful, although not final, in differentiating the two conditions. The spinal fluid findings are so similar that they are of little aid in making the diagnosis.

Brain tumor and abscess of the brain must also be differentiated; and these conditions have proved a stumbling-block to very able neurologists. Brain tumor is comparatively rare in children. It is usually afebrile, and a roentgen-ray examination should be of value. A choked disk is, of course, much more common in this condition than in encephalitis. The spinal fluid findings vary greatly in brain tumor, so that in this condition they will not be of so much diagnostic value. Abscess of the brain is rare and is usually accompanied by some demonstrable primary focus. In abscess of the brain, the blood culture is frequently positive.

Syphilitic conditions of the central nervous system must always be considered, and they may be ruled out with a fair degree of assurance by the result of the Wassermann test and the colloidal gold test of the spinal fluid. Cerebral hemorrhage, thrombosis or embolism are of less importance in children than in adults. Of course, a cerebral embolism with an endocarditis occasionally occurs in children, and must be differentiated from both poliomyelitis and encephalitis. Uremic conditions must also be considered, and must be ruled out by the urinary examination, and the test of the kidney function.

The diagnosis from various acute forms of purulent meningitis is usually easy clinically, and may be made absolutely by the examination of the spinal fluid.

Of course, the diagnosis must also be made from acute infections in children, in which convulsions develop, and sometimes stupor, such as typhoid, pneumonia, and severe gastro-enteritis; but these can usually be easily ruled out by finding the underlying cause and by various laboratory tests, as well as by a test of the spinal fluid, which, in these conditions, is usually normal.

Report of Cases

Case 1.—F. A., a girl, aged 5 years, with no history of previous illness, had a gradual onset about October 1, 1920. The child was listless and apathetic, slept a great deal, vomited once; had no headache; the bowels were constipated, and there was loss of sphincter control of both bowel and bladder about October 12. When examined, October 20, she was apathetic, the temperature was normal, respirations were regular, and the pulse varied in rate from time to time; the pupils were equal, and reacted to light; there was no stiffness of the neck nor Kernig sign, the reflexes were present and equal, and there was no paralysis. Lumbar puncture gave about 35 cc. of clear fluid under pressure. This showed a slight increase in mononuclear cells, and also in albumen and globulin. The reduction of Fehling's solution was normal. Soon after this the child refused to speak. This lasted for about two weeks. Then speech returned, and the incontinence finally cleared up about the middle of November. At last reports she showed complete recovery physically, but was still nervous and irritable.

Case 2.—G. L., a girl, aged 5½ years, gave a history of poliomyelitis in 1916, with paralysis of right arm and leg. The paralysis of the leg had nearly cleared up but that of the arm remained. The onset of the disease occurred the latter part of January, 1921, with headache, vomiting, convulsions, and a temperature up to 103 F. When seen February 14, she was crying out constantly and apparently could not see. The temperature was still around 103 F., and 20 cc. of clear fluid was withdrawn. This fluid showed 460 cells—90 per cent. mononuclears, albumin and globulin + + +, and Fehling's solution normal. After the puncture, the temperature subsided. The child screamed almost constantly for several days, and then began crying real tears. She paid no attention to questions, indeed, did not speak at all. These symptoms lasted about ten days, then the condition improved. The child began to speak but was at first slow in remembering and in speaking. The sight returned. The condition was very satisfactory until March 13, when the child was taken for an automobile ride. In the afternoon, she had a convulsion and became unconscious, Cheyne-Stokes respirations developed, with feeble, rapid pulse, and marked cyanosis. Lumbar puncture was done in the afternoon, showing clear fluid, greatly increased pressure, 35 cells, albumin and globulin + +, and Fehling's solution normal. Next day the patient seemed practically well, but continued to have a slight rise of temperature for several weeks. She is now reported as entirely recovered.

The next case has been previously reported, but is included now on account of the sequelæ.

Case 3.—E. M., a girl, aged 14, gave a history of measles, and paralysis of the left arm and leg, probably poliomyelitis, which developed some years before and had practically cleared up. The present illness developed rather slowly, beginning about January 12, with apathy, tremors and subnormal temperature (96 F.) the chief symptoms. She had grown progressively worse, and when examined, January 16, had the appearance of being in a very serious condition. Her temperature was still 96 F., she was sweating profusely, catatonia had developed, and there were marked tremors. She was extremely apathetic and apparently in a semistuporous condition, but she answered questions correctly, though very slowly and in a monotonous voice. The neck was moderately stiff and there was a question as to the Kernig sign, since there was generally spasticity of the muscles. The knee jerks were equal and exaggerated. The pupils were equal and responded to light. There were no disturbances of vision. Lumbar puncture revealed clear fluid under increased pressure, a moderate increase in cells, 90 per cent mononuclear, albumin and globulin + +, and Fehling's solution normal. For about a week following, the temperature was slightly elevated; the neck was rigid and the patient was troubled with insomnia. Thereafter she slept for about seventeen hours, and then began to improve rapidly. She returned to school and seemed perfectly well until late in June, 1920. During the summer of 1920 she became very indifferent, disliked doing anything about the house, or taking care of her person, and was extraordinarily slow in accomplishing any task, even a very simple one. When seen in October, memory was good, but she spoke in a monotonous voice, and very slowly, and the face was expressionless except for frequent silly giggles. The muscles were spastic and reflexes exaggerated, but she was able to walk about, though very slowly and with a spastic gait. This condition became progressively worse, and when seen in May, 1921, she was unable to walk without being practically carried. She could not sit down or rise from a chair without a great deal of assistance; there was a constant tremor of the hands and feet; the muscles of the whole body were extremely spastic; she had difficulty in swallowing, and the voice was monotonous and slow; but memory was still good, and she seemed to have considerable insight into her condition. She still had a rather silly laugh, but it seemed to come from difficulty in muscle control rather than from lack of intelligence. She had obstinate constipation and there was frequency of micturition. Such a development after four or five months of apparent recovery is a striking commentary on the sequelæ which so often follow this disease. The final outcome of this case will be a matter of great interest.

SEQUELAE

That the effects of the disease may be prolonged for many months and that symptoms may develop after a period of apparent return to health has been pointed out by Abrahamson, Happ and Blackfan; Leahy and Sands; Happ and Mason and others. I have tried as far as possible to follow up our recovered cases at varying intervals.

On account of the great interest centering around the sequelæ, I have included those in adults as well as in children. They seem to develop more frequently in adults than in children. Up to the end of March, sixty-three cases, or 39.3 per cent. of the recovered cases, showed sequelæ for varying lengths of time after the subsidence of the acute symptoms. In these cases, twenty-one patients were less than 15 years old, and forty-two were adults. A part of this difference may be due to the greater ease in eliciting in adults certain conditions, such as headache, mild grades of mental deterioration, or lack of power to concentrate. It is, of course, too early to decide as to the permanence of the sequelæ. However, three out of ten recovered patients, in 1918 are still showing some results of the disease. And it is possible that some of the more recent cases that now seem to be complete recoveries may develop sequelæ in the next few months.

Like the symptoms, the sequelæ are also varied, and it is difficult to group them in any very satisfactory way. Moreover, one patient often shows several sequelæ.

In a general way they may be loosely classified as:

Weakness or paralysis: Adults, 8; children, 4.

Tremor, choreiform movements, spasm; Adults, 6; children, 3.

Pains in body or limbs: Adults, 2.

Headache: Adults, 8.
Dizziness: Adults, 3.
Nervousness: Adults, 5; children, 6.
Change in disposition: Adults, 1; children, 3.
Insomnia: Adults, 3; children, 2.
Drowsiness: Adults, 1; children, 3.
Speech defects: Adults, 6; children, 3.
Defect of Eyes: Adults, 9; children, 4.
Change in mentality: Adults, 14; children, 10.

The changes in mentality are of great interest. In children there are usually indications of mental deterioration—the child is demoted in school, when previously it had made good progress. In adults there is loss of memory and of ability to concentrate, in the milder cases, and sometimes mental depression. In one instance a young woman committed suicide during convalescence. One man was at a hospital for the insane for some months, but was finally paroled. A woman has been at such an institution for over a year.

It is interesting to compare these frequent, diverse and serious sequelæ with those of poliomyelitis and epidemic meningitis. In the former, the sequelæ are certainly sufficiently grave, but they are confined almost entirely to flaccid paralyses. In meningitis only a very small percentage of sequelæ occurs. The most serious is deafness, and this occurs in only 3 or 4 per cent of cases. Blindness, due practically always to a panophthalmitis, is still more rare. While a popular opinion exists that mental defects are often due to meningitis, a careful study of recovered cases of meningitis fails to corroborate this opinion.

Relapses after comparatively short periods of improvement occasionally occur. In one instance, already referred to, there was a second attack, at the expiration of a year of good health, which proved fatal.

MORTALITY

The mortality reported by various writers has varied greatly. In France it has been around 25 to 30 per cent. and in England as high as 50 per cent. In this country, Abrahamson reports a mortality of only 10 per cent., and Happ and Mason a mortality of only 7.4 per cent., while others have reported a mortality as high as 40 per cent. Smith gives a mortality of 29 per cent. In our series of cases, it has been about 28 per cent.

TREATMENT

The general treatment is of great importance and consists, in brief, in general hygienic measures, keeping the patient comfortable, quiet, and well nourished. Feeding by gavage may be necessary. There must be careful attention to the elimination, as constipation is often persistent and obstinate, and retention may occur. While numerous drugs and intraspinal injections have been used, the degree of success attending any of these measures has been small. Lumbar punctures for the relief of pressure have seemed of value to me and are recommended by many writers. The patient should be carefully watched during convalescence, and one should not be too hopeful of a complete cure until some months have passed.

For bibliography see the original article.

A STUDY OF FOUR HUNDRED AND FIFTY CASES OF EPIDEMIC ENCEPHALITIS

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Introduction. Since epidemic encephalitis was first recognized, an enormous literature has developed in continental Europe, England and the United States.

In this paper no effort will be made to review this literature, as our object is simply to present the outstanding features that have developed in the study of a rather large number of cases.

These cases were seen beginning in the fall of 1918 and during the following five years up to 1924. The 1924 cases, 81 in number, have not been included, as we wish to emphasize somewhat the after effects of encephalitis and it is still too early to report on the sequelæ of these cases.

In the fall of 1918 we first realized that we were beginning to see cases of a type that was new to us. Since 1910 the meningitis division has been studying cases of meningeal involvement, the different kinds of meningitis, poliomyelitis, meningism occurring in the various acute infections and other rarer conditions involving the central nervous system. This experience gave us a fairly good background on which to study this interesting disease.

At first we did not recognize that the cases were epidemic encephalitis. We were misled by the reports in the English literature, that the spinal fluid findings were negative, and also by the fact that the English reported that the cardinal symptoms were lethargy, asthenia and cranial nerve palsies. With rare exception, the spinal fluids in our cases have not been normal. While the triad of symptoms described by the English is very characteristic when present, many cases do not present all three and some not even one of these symptoms. As time has passed it has been recognized more and more that cases of epidemic encephalitis present a widely varied symptomatology. The term encephalitis lethargica seems to us very unfortunate, since it emphasizes a symptom which is frequently not present. It is very difficult to convince a patient or his family that he has encephalitis lethargicia when insomnia and restlessness are the outstanding symptoms.

The following, Tables I, II and III, show the distribution of cases by year, season, sex and age:

TABLE I

DISTRIBUTION BY YEARS AND SEASONS

Year.	Jan. Feb. Mar.	Apr. May. June.	July, Aug. Sept.	Oct., Nov., Dec.	Total.
1918	0	0	1	10	11
1919	26	4	9	29	68
1920	65	19	11	16	111
1921	49	25	25	9	108
1922	18	34	8	11	71
1923	45	17	14	6	82
Total	203	99	68	81	451

From the above table it will be seen that the greater number of cases occur in the first three months of the year and the smallest number in the summer months.

TABLE II.
DISTRIBUTION AS TO SEX

Year	Male	Female
1918	5	6
1919	52	16
1920	68	43
1921	60	48
1922	34	37
1923	45	37
Total	264	187

From the above table will be seen that approximately 59 per cent. are males and 41 per cent. females, or a little less than a ratio of 3 to 2. The preponderance of males over females is slightly less than in epidemic meningitis and poliomyelitis. In these diseases about 50 per cent. more males than females are attacked.

It will be seen from Table III that of the 451 cases 234 will more than fifteen years of age and 217 cases were less than fifteen years of age. This is a larger proportion of cases in childhood than is reported by most workers. But we see an unusually large number of cases in children because many physicians in the city are accustomed to call us to help them in the diagnosis of meningeal conditions in children.

TABLE III
AGE DISTRIBUTION

Age.	1918.	1919.	1920.	1921.	1922.	1923.	Total
Under 6 mos.	0	3	3	2	2	0	10
6 to 12 "	2	2	2	2	3	1	12
1 to 2 years	0	5	11	11	5	6	38
2 to 5 "	1	10	8	13	8	8	48
5 to 10 "	3	7	21	19	10	9	69
10 to 15 "	1	7	13	5	5	7	38
15 to 20 "	1	7	5	9	6	10	38
20 to 30 "	2	10	22	24	10	12	80
30 to 40 "	0	9	17	9	6	11	52
40 to 50 "	1	7	8	7	5	8	36
50 to 60 "	0	1	0	7	9	6	23
over 60 "	0	0	1	1	1	4	7
							451

For some time we have felt that cases in children were frequently overlooked. If we have made an unusually large percentage of mistakes in the diagnosis of epidemic encephalitis in young children we have made it consistently. Up to the middle of May, 1921, when 274 cases had been studied they were equally divided between patients more than fifteen years of age and those younger. Our findings are corroborated by the work of Happ and Mason, whose age distribution of cases is almost the same as ours.

In contrast to this, about 65 per cent. of cases of epidemic meningitis and about 90 per cent. of cases of poliomyelitis are less than ten years of age.

Symptomatology. While the chief symptoms are those referable to the central nervous system, there are also symptoms, such as fever, which is usually low and irregular, vomiting and malaise, which may be caused by a general toxemia.

The onset is usually gradual but in a certain percentage of cases it may be abrupt. It is more likely to be sudden in children than in adults. The symptoms referable to the central nervous system are of the greatest possible variety and vary widely in the same patient during the course of the disease, which is often greatly prolonged. This indicates that we are dealing with a virus which may involve any part or combination of parts of the central nervous system and which may progress from one part to another during the illness. This is in marked contrast to the action of the virus in poliomyelitis which attacks suddenly and has usually progressed to the maximum within five to seven days. In poliomyelitis the action of the virus is far more selective, and the greatest damage is done to the cells of the motor area, especially to the cells of the anterior horns of the spinal cord.

We have made no attempt to divide our cases into groups. The multiplicity of symptoms necessitates so large a number of groups that the classification becomes too unwieldly for the use of the general practitioner. Moreover, on account of the progress of the disease the same patient would often fall into any one of several groups, depending on the time at which he was seen.

So much has been written on the symptomatology of epidemic encephalitis that our discussion will be brief. We do wish to emphasize the fact that quite often the symptoms are mild and in other instances different from those originally considered pathognomonic. We feel that the diagnosis is missed in many instances because the physicians are looking for lethargy, asthenia and cranial nerve palsies. While extreme lethargy and somnolence are very characteristic when present, a fairly large group of cases suffer from restlessness and insomnia. In some patients both somnolence and insomnia are present at different stages of the disease.

Ocular disturbances of various kinds are frequent occurrence. Attacks of diplopia or blurring of vision are sometimes the earliest distinctive symptoms. These attacks may last for only a short time in the early stages or they may occur at intervals for a considerable period. Sometimes the diplopia or blurring is fairly continuous over most of the illness. Loss of vision, usually temporary, is occasionally seen. Other phenomena that are sometimes noted are: Ptosis, either unilateral or bilateral; strabismus, nystagmus and hippus. In one instance there occasionally took place convulsive twitchings of the eyes which then snapped quickly to the left, and remained fixed for two or three minutes. These seizures occurred at frequent intervals during the height of the diseases, but disappeared entirely as the patient recovered. In another case the eyeballs performed in succession a curious, nearly complete rotation, with the pupils looking inward. While one eye was in motion the other eye remained quiet, but there was more or less twitching of the lips and face muscles on the same side. While a large percentage of cases show a normal fundus, a slight haziness of the disc has been seen in a certain number of cases. Various degrees of optic neuritis with or without choking has been observed less often. Sometimes in late cases there is secondary optic atrophy.

In addition to the lethargy or excitability which are very common symptoms, and have already been referred to, delirium is not infrequently present. The delirium may last for a considerable time, or may occur at frequent intervals, lasting only for a few minutes. In other instances the patient is acutely maniacal for varying lengths of time. Catatonia is occasionally seen.

Sensory Disturbances. Headache is an almost constant symptom. Lancinating pains due to involvement of the posterior nerve roots are fairly common. These affect most commonly the arms and legs, but the thoracic muscles are sometimes involved. Numbness or tingling are less frequent symptoms. Occasionally there is loss of sensation, involving one or both legs and lower part of the trunk, resembling a hemitransverse or a tranverse myelitis.

Trophic Disturbances. These are rare but do occur during the early or later stages of the disease. Repeated ulcerations of the cornea were seen in one patient beginning during the comparative early stages and lasting for several months. In another case there was an extensive furunculosis late in the disease, and another case, also late, there were frequent attacks of stomatitis, thought to be trophic in origin. In one case the teeth decayed very badly after the attack of encephalitis and in another there was alopecia.

Motor Symptoms. One of the most striking of the motor symptoms which is found in a certain proportion of the cases is hypertonicity of the muscles. This is shown in the masklike expression of the face, in the general muscular rigidity and the slow movements. The rigidity of the neck due to this general hypertonicity must be sharply differentiated from the rigidity due to inflammation of the meninges. Muscular twitchings are a common phenomenon. They may be of several varieties. Myoclonic contractions may involve a single muscle or any group of muscles. Tetanic spasms are less common. Occasionally a persistent hiccough is seen. Tremors of various types frequently occur. They may be very fine or assume a choreiform or athetoid character. Convulsions are common in children. Paralysis or pareses are very frequent. Involvement of the oculomotor apparatus has already been referred to. Weakness or paralysis of the muscles of deglutition is comparatively rare. Facial palsies may be either of the central or peripheral type. Practically any form or combination of forms of extremity paralysis or paresis may occur, and this is usually of the spastic type, although not invariably so. The disturbances of speech are a little difficult to group. Some in which there is an obvious dysarthria are probably due to disturbed function of the motor mechanism. In other instances there is a great delay in answering questions. Speech may be hesitant, slow or indistinct.

The reflexes, superficial as well as deep, may be normal, diminished or exaggerated and may be equal or unequal. An Argyll-Robertson pupil is often seen, while loss of accommodation reflex is less common.

The Babinski and confirmatory signs are often present, and the Brudzinski sign is common. The Kernig sign is frequently present. Both patellar and ankle clonus are occasionally obtained.

Other symptoms may be noted, most of these evidences of a systemic infection. Fever is a very constant sign. It is usually low and irregular. Occasionally it is subnormal for a part of the illness. Vomiting is fairly common early in the disease. An obstinate constipation is quite characteristic. Jaundice is sometimes seen but is rare. Bladder disturbances are not infrequent. There may be either incontinence or retention. Hematuria has occurred in two or three instances. Disturbances of the respiratory rhythm occasionally occur.. They may take the form of very rapid respiration of forced inspiration. The Biot type is fairly common. Cheyne-Stokes is rare except in the terminal phases. Profuse sweating is a rather characteristic sign and occurs not infrequently. Epistaxis has been noticed as an early sign but has been seen in a very few cases.

It may be well to say a few words about the symptomatology in children, as it is our impression that the diagnosis is often missed. In many cases the symptoms are milder than in adults; in others they are more atypical. Probably the most common form in children is that resembling an early tuberculous meningitis. In these cases the diagnosis can be made only by repeated examination of the spinal fluids. In a certain number of cases the children show symptoms quite unlike those we consider characteristic of epidemic encephalitis. The following is a case in point:

G. L., a girl, aged five and a half years, was seen, February 14, 1921. She had had poliomyelitis in 1916, with paralysis of the right arm and leg. The paralysis of the leg had nearly cleared up. The present illness had begun three weeks before with headache, vomiting, convulsion, delirium and irregular fever reaching 103°. No lumbar puncture had been done. On February 14 she was crying out constantly in a meaningless fashion and was apparently blind. A lumbar puncture was done, yielding 20 cc. of clear fluid with characteristic findings. The temperature began to subside after the puncture but the screaming persisted for several days and then she began weeping. This lasted for two or three days. Then the condition began to improve. The sight returned, the child began to speak rationally, but was slow in remembering and speaking. The improvement continued until March 13, when after an automobile ride she had a convulsion and became unconscious. Cheyne-Stokes respiration developed, the pulse was feeble and there was cyanosis. Another lumbar puncture was done that afternoon, yielding a clear fluid under increased pressure. This was followed by immediate improvement, and the next day she seemed as well as she had been before the convulsion. The improvement continued, although for several weeks she had a slight rise in temperature every second or third day. Three years later she was reported to be in excellent condition with no after effects.

Differential Diagnosis. The symptomatology of epidemic encephalitis is so varied that a complete diffential diagnosis would necessitate a discussion of a very large number of diseases of the central nervous system and many of the acute infections. We shall attempt to discuss the differentiation from only the most important of these.

Tuberculous meningitis is probably the disease from which epidemic encephalitis is most often to be differentiated. The diagnosis may be absolutely made by examination of the spinal fluid and therefore time will not be taken to discuss the different points of clinical diagnosis as they are often misleading. Diplopia, for example, occasionally occurs in tuberculous meningitis. The finding of tubercles in the choroid also makes the diagnosis of tuberculous meningitis certain. The differentiation from the other forms of meningitis is less difficult, but even here mistakes are sometimes made. In purulent meningitis diplopia or disturbances of vision sometimes occurs. A lumbar puncture, of course, immediately makes the diagnosis.

The differentiation from poliomyelitis is usually made, especially from the spinal form. From certain forms that have been described in the past as the encephalitic type of poliomyelitis, the diagnosis at present is a matter of personal opinion. Certain authorities consider that cases previously diagnosed as the encephalitic type of poliomyelitis were really cases of epidemic encephalitis, and it is possible that the classification of poliomyelitis will have to be revised.

The differential diagnosis from brain tumor or abscess of the brain is often made only after extensive radiographic studies or at autopsy. These conditions are so infrequent that the percentage of error in a large series of cases is exceedingly small.

Syphilis of the central nervous system in some cases can only be differentiated by laboratory aid, the Wassermann test.

Cerebral hemorrhage and thrombosis are often mistaken for epidemic encephalitis and *vice versa.* The differential diagnosis is often extremely difficult, especially from cerebral thrombosis. A hemorrhagic fluid makes the diagnosis of cerebral hemorrhage very probable, although blood-tinged fluids may be found in cases of encephalitis.

Cardionephritic conditions, especially in the pre-uremic stages, frequently resemble epidemic encephalitis. Studies of the blood chemistry and determination of renal function will prove the determining factors in establishing final diagnosis in this type of case.

Certain forms of mental disease, dementia precox, for instance, often present signs and symptoms similar to those of epidemic encephalitis. The case may have to be followed for some time before a definite diagnosis can be made.

Mention must also be made of encephalitis following acute infections, especially measles. The history of a recent acute infection will be the determining factor in making the diagnosis as the symptomatology and spinal fluid findings may be similar in the two conditions.

Laboratory Aids in Diagnosis. The blood examinations is not at all characteristic in epidemic encephalitis. The blood count usually shows a low leukocytosis from 10,000 to 15,000 but sometimes a little higher. The blood cultures are sterile. The analysis of the spinal fluid is the most diagnostic laboratory aid. The spinal fluid is usually clear and increased in amount; sometimes it is slightly hazy and it may at times be yellowish. There have been instances where we obtained a blood-tinged fluid. The cells are usually slightly or moderately increased, the cell count being ordinarily less than 100, with a preponderance of mononuclears. Occasionally there may be a larger increase in the number of cells up to 1000 or more. A preponderance of polymorphonuclears may at times occur. With but few exceptions we have found a slight or moderate increase in albumin and globulin content. Fehling's solution is promptly and well reduced, denoting a normal or even more than a normal amount of sugar in the spinal fluid. Of late we have used the quantitative method of determining the amount of sugar in the spinal fluid, and we have found the figures to range between 50 mg. and 100 mg. of sugar per 100 cc. Bacteriologically the fluid is sterile. These findings, while characteristic, are by no means pathognomonic, since similar findings are obtained in poliomyelitis, in some instances of syphilis of the central nervous system and early in tuberculous meningitis.

It may not be amiss to discuss in more detail the importance of the sugar content of the spinal fluid. Certain writers have stated that an increase in the sugar content is pathognomonic of epidemic encephalitis. While it is frequently found in epidemic encephalitis, it is also found occasionally in poliomyelitis, syphilis of the central nervous system and even in meningism. The determination of the spinal fluid sugar is of most value in differentiating tuberculous meningitis. In this disease, when well advanced, the spinal fluid sugar is usually, but not invariably, greatly diminished.

Mention should also be made of the Wassermann test which should always be done when there is any suspicion of syphilis. The colloidal-gold reaction is not so important, since luetic and paretic curves are fairly common findings in epidemic encephalitis.

In differentiating tuberculous meningitis from epidemic encephalitis, if the routine spinal-fluid examination is not definite, recourse must be had to guinea-pig inoculation.

In a few instances where the diagnosis was unquestionably epidemic encephalitis we have found a normal spinal fluid. Other writers have reported a fairly large proportion of normal spinal fluids. However, a study of the spinal fluid analysis in many of our leading hospitals has convinced us that such examinations are often incomplete.

Pathology. Epidemic encephalitis belongs to the class of inflammatory diseases in which also are included poliomyelitis, syphilitic lesions of the central nervous system and trypanosomiasis. While these different diseases have, broadly speaking, certain characteristics, the cases in a given class differ so widely that it is difficult, if not impossible, to accurately diagnose by a study of the pathology alone, the less typical cases.

The meninges are usually described as showing only slight changes—an increase in the cellular content, particularly in the neighborhood of the blood vessels of the pia-arachnoid. The cerebral cortex is generally normal, except for congestion of the vessel of the leptomeninges. In the brain substance the changes are most marked in the basal nuclei of the brain, the upper part of the pons and peduncles, the gray matter of the floor of the fourth ventricle and the aqueduct of Sylvius. The changes in the medulla and cord are often reported as less pronounced, though observers have noted the same changes occurring in the upper section of the cord. This was certainly observed in one of our cases, an adult, which came to necropsy. (Reported in the *International Clinics,* vol. 2, series 29.)

The lesions are generally described as consisting of four kinds:

1. Infiltration of the walls of the small vessels with lymphocytes and plasma cells.

2. Foci of interstitial and parenchymatous infiltration with round cells. In this reaction neuroglia cells may take part.

3. Lesions of the nerve cells—usually not so extensive as in poliomyelitis and with less neuronophagia. These lesions of the cells usually occur when the inflammatory process takes place in the gray matter, but they may develop in the absence of an inflammatory reaction. Such is the case with regard to the cells of Purkinje in the cerebellum where inflammatory changes are almost entirely absent.

Foci of perivascular hemorrhage. The vessel walls are usually not necrosed.

In connection with the statement that lesions of the cells may occur in regions where there is no evidence of inflammatory reaction, it is interesting to recall that Abramson, in a very excellent study of the pathology of poliomyelitis made at the Research Laboratory during the epidemic of 1916, brought out the same fact in regard to the lesions of poliomyelitis.

Treatment. While many methods of treating epidemic encephalitis have been tried, there has been such a lack of uniformity in results that it is safe to say that at present there is no specific treatment. The majority of observers have agreed that lumbar puncture is beneficial. Some cases seem to do better with repeated lumbar punctures; others are apparently unaffected. None of the cases we have observed have suffered ill effects from lumbar puncture. No definite rule as to the repetition of lumbar puncture can be followed. We have been guided by the clinical condition and the reaction to lumbar puncture, also by pressure symptoms and amount of fluid removed. In some cases the beneficial effects following lumbar punctures have been striking. The following case illustrates this:

N. P., a man, aged twenty-nine years, was seen, January 1, 1923. Past history and family history are unimportant. Present illness developed rather suddenly on December 25, with chills, headache and fever, 103.5°, but without vomiting. On January 1 the patient still had a temperature of 103°, stiffness of the neck, slight ptosis of the lids and double vision. Patellar reflexes were slightly exaggerated. There were signs of bronchitis in both lungs. The patient was quite nervous and very excitable. The headaches and excitability were gradually getting worse while the diplopia was diminishing. Physical examination was otherwise negative. Lumbar puncture was done and 40 cc. of clear spinal fluid under markedly increased pressure was obtained. On examination the spinal fluid showed a large increase in cells; albumin and globulin, + +; sugar was normal. The patient apparently derived so much relief from the puncture that one was done every other day, seven in all. On January 14, twenty days after the onset, the patient was apparently all right except for slight weakness. He made an uneventful recovery without any sequelæ, and is at work and entirely well today. This is apparently an instance where repeated lumbar punctures proved beneficial.

Except for lumbar puncture, the treatment is symptomatic. The symptoms are so varied that the length of this paper will not warrant a full discussion.

The mortality in our series of cases is given in Table IV.

Course of the Disease. When epidemic encephalitis first appeared it was considered a somewhat acute infection of the central nervous system, more or less like poliomyelitis. As time has gone on and cases have been studied further this opinion has been revised. Now we know that in many instances it assumes a very chronic course with periods of remissions and exacerbations. Sometimes relapses with acute symptoms occur after one or more years of apparently good health. It is not certain whether these are due to a lighting up of the old infection or to a new infection. One of our patients died of a second attack after being apparently well for a year. In other instances a year or more after the patient has apparently recovered, a slowly progressive condition, for example, a Parkinsonian syndrome develops. This is usually considered a sequela, but the opinion has been expressed that

TABLE IV.—MORTALITY.

Age.	1918.	1919.	1920.	1921.	1922.	1923.	Total	Mortality as to age, per cent.
Under 6 months	..	..	1	1	..	..	2	20.0
6 to 12 "	1	..	1	1	1	..	4	33.3
1 to 2 years	..	3	7	4	2	1	17	45.8
2 to 5 "	..	3	3	2	3	4	15	31.2
5 to 10 "	..	3	9	2	5	..	19	27.5
10 to 15 "	..	1	3	..	..	1	5	13.4
15 to 20 "	1	2	3	..	..	1	7	18.4
20 to 30 "	..	2	8	4	5	2	21	26.2
30 to 40 "	..	1	3	3	..	1	8	15.3
40 to 50 "	..	3	3	1	2	1	10	27.0
50 to 60 "	..	1	..	4	6	4	15	68.2
Over 60 "	..	..	1	..	..	3	4	57.1
Total deaths . .	2	19	42	22	24	18	127	
Total Number of cases	11	68	111	108	70	83	451	
Mortality	Per cent. 18.2	Per cent. 27.9	Per cent. 37.8	Per cent. 23.8	Per cent. 34.3	Per cent. 21.9	Average mortality	28.2

this is a secondary attack or a relapse. In other cases these chronic conditions and progressions follow the more acute stages without any marked improvement in the patient's condition. In general, the course of the disease is similar to that of syphilis of the central nervous system. It must not be forgotten, however, that a fairly large number of cases have remained well over a period of four or five years. The chronicity and variability of this disease is well brought out by the following case:

The patient, H. F., a female child, aged two years, was taken ill suddenly on August 18, 1923, with fever, 102°, and profuse sweating. The child was also gradually becoming progressively drowsy. The family and past history were negative. The physical examination on the fifth day of her illness showed the child to be acutely ill and apathetic. The temperature was 101°; pulse, 90; respiration, 20. There was definite rigidity of the neck. There was double internal strabismus. All the deep reflexes were normal, but the abdominals could barely be elicited. The Kernig, Brudzinski and Babinski signs were all negative. There was a definite weakness of the muscles of the back. A diagnosis of meningitic type of poliomyelitis was made at that time. Lumbar puncture was performed and 30 cc. of clear fluid under increased pressure was obtained. On examination the fluid showed a moderate increase in cells, 98 per cent. of which were mononuclears, a slight increase in albumin and globulin and a prompt and good reduction of Fehling's solution. On smear and culture no organisms were found.

October 2, 1923: There was a marked generalized rigidity, particularly of the neck. The strabismus had practically cleared up. The child also presented a typical picture of the Parkinsonian syndrome. There was also Parkinsonian tremor of the hands. It was now obvious that the child was suffering from encephalitis. Lumbar punctures performed at that time yielded 30 cc. of clear fluid under marked increased pressure.

October 17: Child still had Parkinsonian syndrome but to a lesser degree. The tremor was less marked and the child was able to walk a little. The neck was still rigid. Her general appearance was much brighter. On lumbar puncture 45 cc. of clear fluid were obtained.

June 12, 1924: The child was very much improved, but the Parkinsonian appearance was still quite obvious. There was neither rigidity of neck nor tremor of hands.

April 1, 1925: The child showed marked improvement. She was well nourished, bright and cheerful. The Parkinsonian features were disappearing, though her speech was still slow and the facies slightly masked. Her teeth were badly decayed. There was now a new complicating feature—a definite right hemiparesis of the lower motor neuron type as all her deep reflexes on that side were absent. She was unable to walk except when supported and guided. She also had slight inconstant tremor of her right hand.

This case illustrates a number of very interesting points. In the first place it shows how difficult it is sometimes to make a diagnosis of encephalitis in children. This would have been considered a case of poliomyelitis were it not for the Parkinsonian syndrome that developed two months after the onset of her illness. The chronicity of this disease is also very clearly brought out. The clinical picture has changed a great deal during the different periods of a two-year illness, indicating the involvement of different foci each time. The Parkinsonian syndrome, for instance, was a rather early complication, while later in the course of her illness a right hemiparesis developed as the Parkinsonian picture was clearing up.

We cannot, therefore, consider any of these cases as completely recovered or the sequelæ as permanent until many years have elapsed. It will, indeed, be very interesting and instructive to study a number of years from now the children who have had encephalitis.

Sequelae. It is difficult to obtain a very adequate idea of the sequelæ in epidemic encephalitis. Those who work in neurologic clinics are likely to be unduly impressed with the frequency of sequelæ, since patients with sequelæ go to clinics, while those with complete recovery do not. To obtain a true idea of the frequency of sequelæ it would be necessary to follow a large number of cases for a long time. In the first place, certain patients have symptoms lasting for a year or more, which eventually disappear. For instance, one patient was blind for a year but eventually recovered his sight. Two had change of disposition for about a year and one of them did poorly in school, but both eventually made complete recoveries. In several other instances there were sequelæ lasting several months which eventually disappeared. On the other hand, other patients are in apparently good health for some time, and then develop symptoms which may be considered sequelæ or recrudescences, apparently of a progressive character. Care should, of course, be taken to ascertain that the patient was free from abnormality before the illness. A rather large percentage of our cases had previously suffered from some defect or from some acute infection of the central nervous system before developing epidemic encephalitis. For instance, 2 were definitely mentally defective before the illness; 1 had Little's disease and had developed epilepsy; another had had epilepsy for several years; 1 had had chorea; 3 had had poliomyelitis; 1 had had epidemic meningitis; 1 died from epidemic meningitis while convalescing from epidemic encephalitis.

Of our 451 cases, 127 died. Of the 324 surviving, 36 for one reason or another could not be followed up, leaving 288 cases which were fairly adequately investigated. It was found that 78, or 27 per cent., showed definite sequelæ, while the remainder were carrying on their usual occupations, or in case of children were progressing in school satisfactorily, and to all intents and purposes showed no evidence of abnormality. Many patients showed several residual defects. Although the number of cases was nearly equally divided between those more than, and those less than, fifteen years of age, it was noted that there were 50 with sequelæ in those more than fifteen years of age and only 28 in the other group. The fact that half of our cases were in children accounts to some extent for out percentage of cases showing sequelæ being less than the percentage of other workers.

Table V shows the distribution of the sequelæ:

A study of the classification shows that the greatest number of sequelæ were mental. The next largest group was of motor origin. It is not within the scope of this paper to classify exactly the various types of mental changes. In some instances they were so slight as to be hardly noticeable. In other instances there was a severe mental deterioration that was apparently progressive. How permanent some of the milder sequelæ will be, it is at present impossible to estimate. We have already mentioned that in several instances sequelæ lasting for a year or more eventually disappeared.

TABLE V.

	A. MENTAL	
I.	Blunting of intelligence	30
II.	Nervousness and irritability	13
III.	Defective memory	5
IV.	Changes in character or in disposition	14
V.	Mania or psychosis	2
	B. TROPHIC	6
	C. ENDOCHRINE	5
	D. SENSORY	
I.	Headaches	11
II.	Neuritic pains	6
	E. MOTOR	
I.	Eye changes	
	(*a*) Pupillary	4
	(*b*) Ptosis	2
	(*c*) Strabismus	2
	(*d*) Nystagmus	1
	(*e*) Diplopia	4
	(*f*) Limitation of motion	4
	(*g*) Blurred vision	4
II.	Paralyses or pareses of muscles of face or extremities	13
III.	Parkinsonian syndrome	11
IV.	Tremors, twitches, spasms	11
V.	Epilepsy	1
VI.	Changes in gait	3
	F. MISCELLANEOUS	
I.	Asthenia	9
II.	Digestive disturbances	4
III.	Disturbances of sleep	10
IV.	Speech disturbance	4
V.	Respiratory disturbances	1
VI.	Sphincteric disturbances	1
VII.	Dizziness	3

While we have observed only 11 cases of well-developed Parkinsonian syndrome, certain other cases grouped as asthenia presented some of the features suggestive of an early or mild form of this condition. Perhaps the more common of the motor sequelæ is a paralysis or weakness of some group of muscles of the extremities or face. In one case there was a paralysis of the sternocleidomastoid muscle on one side, thus producing a torticollis.

A general muscular weakness is a rather common after effect. In some cases it is so severe that it would suggest myasthenia gravis, were it not for the history of the previous attack of encephalitis. In the great majority of cases, however, this general asthenia lasts only for several months or a year. This accounts for the small percentage of asthenia as a sequel in our tabulation.

We have also observed some very interesting but rare sequelæ. Four cases of obesity were noted; 1 case developed diabetes mellitus during her convalescence period. It is possible, of course, that this may have been a mere coincidence. We have not noted the report of this complication in the literature, in contrast to diabetes insipidus which has been observed by several writers. Some interesting trophic disturbances have also been observed, such as alopecia, furunculosis, stomatitis, decay of teeth and corneal ulcers.

One patient, an adult, had a very interesting respiratory disturbance. He would get attacks of difficult and labored breathing, lasting from a few seconds to a few minutes. Various types of respiratory difficulties, especially in children, have been referred to in the literature. One patient, a girl aged seven years, showed two years later some of the symptoms that are considered characteristic of multiple sclerosis—nystagmus, scanning speech and staggering gait. The knee jerks were exaggerated. There was, however, no intention tremor.

It is interesting to compare these frequent, diverse and serious sequelæ of epidemic encephalitis with those of poliomyelitis and epidemic meningitis. In the former the sequelæ are certainly sufficiently grave, but they are confined almost entirely to flaccid paralyses. In meningitis only a very small percentage of sequelæ occurs.

For bibliography see the original article.

ENCEPHALITIS ASSOCIATED WITH MEASLES

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Josephine B. Neal and Emanuel Appelbaum

The literature on encephalitis associated with measles is meager and rather indefinite. The ideas with regard to encephalitis have undergone considerable change in the past few years as a result of the intensive study of epidemic encephalitis. There are numerous references to involvement of the central nervous system during or shortly after an attack of measles. In most instances the authors apparently regarded these symptoms as incidental. Only rarely were the symptoms referable to the central nervous system grouped and designated as a definite clinical entity.

It has been recognized for a long time that the various acute infections, as measles, scarlet fever, pneumonia and pertussis, may be followed by encephalitis, most of which have manifested themselves in the form of a cerebral hemiplegia. Particular attention was called to these points by Abt in 1906. While we have seen a few instances of encephalitis following various of the acute infectious diseases, by far the largest number (twelve) have been associated with measles. The first was seen in May, 1918. During the year 1926, particularly, we have seen a comparatively large number (eight). Our experience coincides with that of other clinicians as personally reported to us.

Symptomatology and Diagnosis

The clinical picture is of a highly variable character. The onset of this condition was usually sudden, and developed during or a few days after the attack of measles. In some instances, two weeks elapsed before the appearance of the symptoms referable to the central nervous system. Fever was an almost constant symptom, being present in all but one of our cases. The type of fever was variable. Headache also was almost constantly present. Vomiting was a symptom in only a few cases. Convulsions were present in about half of the cases, and in some instances they were very severe. The most striking of all the symptoms were referable to the mental condition. In some instances there was a mild degree of irritability or apathy. In others there was profound stupor or delirium. Paralysis of various muscles or group of muscles occurred in one-third of our cases. In two instances there was a paralysis of the rectus externus muscle of the right eye, and in one of these cases there was an associated right facial palsy. One patient had a spastic paralysis of the left arm which cleared up in less than a week. The fourth patient had a spastic parlysis of the left arm and a paresis of the left side of the face and the left leg. This patient died after an illness of three weeks, during which period his condition had remained unchanged. Hyperesthesia was fairly common. Muscular twitchings occasionally occurred. General muscular spasticity was a rather common feature. Among the rare symptoms were photophobia, epistaxis, constipation, and retention or incontinence of urine.

The physical signs were chiefly those due to varying degrees of meningeal irritation or increased intracranial pressure. Stiffness of the neck, Kernig's sign, Brudzinski's sign, a bulging fontanel or a positive Macewen sign were noted in a fair proportion of cases. The condition of the reflexes was highly variable. In some instances they were exaggerated and in others they were diminished or absent. In many of our cases, however, the reflexes were normal. The Babinski and its confirmatory reflexes were occasionally present. While ocular symptoms were rare, they were rather striking when present. One patient was apparently blind. Fixed dilation of pupils was present in two cases. There were also two instances of nystagmus and strabismus.

The Spinal Fluid

One or more punctures were performed in every case. The fluids usually came out under increased pressure and were clear in all but two instances, which were contaminated with a few red blood cells. Practically all the spinal fluids showed some evidence of abnormality. There was usually a slight or moderate increase in cells, most of which were mononuclears. In most instances there was also a slight or moderate increase in protein content. It should be noted that occasionally a fluid showed an increase in the cytology without showing a parallel increase in the protein. More commonly however, the protein was increased with or without a concomitant increase in the cell content. The sugar was uniformly normal or high. Smears and cultures were all negative for organisms. The accompanying table shows the spinal fluid analyses.

Differential Diagnosis

It is obvious that all forms of encephalitis present more or less similar clinical pictures. Attention has been called in a preceding paragraph to the association of encephalitis with various acute infections. The diagnosis of our cases of encephalitis associated with measles was based almost exclusively on the manifestations of encephalitis occurring during or shortly after an attack of measles. Indeed, there was nothing in the clinical picture alone to rule out epidemic encephalitis definitely. It may be noted, however, that the onset was more sudden, and, with one exception, the duration was much shorter than is usually the case in epidemic encephalitis.

Report of Cases

Six cases will be described briefly in order to illustrate somewhat the clinical picture and course of the disease.

Case 1.—G. I., a girl aged 6 years, seen March 20, 1926, had been suffering from measles for one week. Two days after the onset of the measles, she was seized with convulsions, which were followed by apathy deepening into stupor. The examination showed the patient to be acutely ill and still in a semistuporous condition. The temperature was 102 F., the pulse 120, and the respiration 28. There was a questionable Kernig sign. The pupils were rather small but reacted to light. The patellar reflexes were exaggerated. The abdominals were absent. The Babinski reflex was negative but there was a positive Oppenheim sign on the left side. Lumbar puncture yielded 20 cc. of clear fluid under moderately increased pressure. There was a large increase in cells, mostly mononuclears. There was a moderate increase in albumin and globulin. The Fehling's reduction for sugar was normal. Both the smear and the culture were negative for organisms. Lumbar puncture was followed by an immediate improvement, especially in the mental condition. At the end of a week the child had practically recovered. This case seems to illustrate a fairly typical clinical picture and course.

Case 2.—I. D., a boy aged 3 years, was suddenly taken ill with headache and vomiting nine days after an attack of measles. He did not have fever but was apathetic. At times, however, he was quite irritable. The bowels were constipated, but there were no bladder symptoms. Physical examination did not show anything striking. He was, however, definitely apathetic, and had a positive Babinski reflex on his left side. On lumbar puncture, 30 cc. of clear fluid were obtained under increased pressure. The fluid showed a slight increase in cells, mostly mononuclears. The albumin and globulin were also slightly increased, and Fehling's reduction was normal. After an illness of a few weeks the boy made a perfect recovery.

SPINAL FLUID EXAMINATION

Case	Amount of Fluid Cc.	Appearance	Cytology	Bacteriology	Albumin	Globulin	Sugar
1	20	Clear	Large increase of mononuclears	Negative	++	++	+++
2	30	Clear	Slight increase of mononuclears	Negative	+	+	+++
3	15	Blood-tinged with web	Mostly red blood cells	Negative	+1	+1	+++
4 (*a*)	30	Clear	3 mononuclears	Negative	++	+	+++ (57 mg.)
(*b*)	..	Clear	12 mononuclears	Negative	+	+	+++
(*c*)	15	Clear	8 mononuclears	Negative	±	±	+++
5	24	Clear	3 mononuclears	Negative	+	..	+++
6	30	Clear	15 mononuclears	Negative	+	+	+++ (84.2 mg.)
7	15	Clear	Normal	Negative	+	+	+++
8	20	Clear	87 mononuclears	Negative	+1	+1	+++
9	20	Clear	Slight increase of mononuclears	Negative Negative	±	±	+++
10	15	Clear with web	Large increase of mononuclears	Negative	+1	+1	+++
11	15	Clear	Normal.	Negative	±	±	+++
12	5	Blood-tinged	Red blood cells		±	±	+++

This was undoubtedly a mild case of encephalitis following an attack of measles. It should be noted that there was an interval of nine days between the measles and the encephalitis.

Case 3.—E. H., a boy aged 7 years, was taken ill suddenly, April 27, 1926, with a headache, convulsions, and a fever of 102 F. Shortly after the onset of this illness he developed a spastic paralysis of the left arm. Six days prior to this illness he had an attack of measles. When we saw him, April 30, he was quite apathetic. His temperature was 100.4 F., pulse 120 and respiration 28. Hyperesthesia was marked. There was a positive Kernig sign on the right side and also a positive Brudzinski sign. The Babinski sign was questionable. There was a definite spastic paralysis of the left upper extremity.

Lumbar puncture yielded 15 cc. of slightly blood-tinged fluid, which showed a moderate increase in albumin and globulin and a normal Fehlig's reduction. Smear and culture were negative for organisms.

May 3, the child had completely recovered. The paralysis of the left arm had cleared up, and he seemed to be perfectly normal in every way.

This case illustrates some interesting points. It shows that one cannot be guided by the severity of the case in determining the prognosis. The child certainly was very ill and yet made an uneventful recovery. It is also obvious that the total duration of the illness may be very brief.

Case 4.—W. F., a boy aged 20 months, had measles January 11, 1926. This was followed by bronchitis, for which he was admitted to a hospital January 23. On January 26 he developed weakness and spasticity of the left upper and lower extremities. After a few days the left arm became definitely paralyzed, and there also developed paresis of the left half of the face. On examination, the child was very apathetic. When aroused, he was irritable. The neck was slightly rigid. The pupils were small and reacted to light. The deep reflexes were normal on the right side and exaggerated on the left side. The abdominals were absent on both sides. There were no pathologic reflexes. There was a spastic paralysis of the left arm and paresis of the left side of the face and left lower extremity. The fundi were slightly hazy. There was a low irregular fever throughout the illness.

Three lumbar punctures were performed. The first two fluids did not show increase in cells; there was a slight increase in albumin and globulin and a normal Fehlig's reduction. Smear and culture were negative for organisms. The third fluid was normal in every respect. Both the spinal fluid and the blood Wasserman test were negative.

The child was ill for about three weeks, during which period his condition remained unchanged. He died, February 11.

Although the clinical course was not by any means stormy, the result was fatal.

Case 5.—T. S., a boy aged 2½ years, seen January 26, 1926, had developed measles on the 15th. Five days later meningeal symptoms developed. These consisted of severe convulsions, vomiting and fever up to 105 F. There was also apathy and incontinence of urine.

When examined, January 26, there was no stiffness of the neck, Kernig sign or Brudzinski sign. The pupils were equal and reacted to light, but the child was apparently blind. He was also unable to speak. There was a right facial palsy and also a paralysis of the right external rectus muscle. The deep and superficial reflexes were abolished and there were no abnormal reflexes. The temperature was 105 F., the pulse 120 and the respiration 40.

During the examination, convulsions occurred. These involved both arms and the face, but not the legs. Several spinal fluids were examined during the illness. The results were normal except for a slight increase in protein. The child died, January 27.

This case illustrates an unusually severe type of the disease.

Case 6.—S. R., a boy aged 5 years, had suffered from an attack of measles, from which he had made a good recovery and had been well for three days. On the fourth day of his convalescence (May 3, 1926) he suddenly had a convulsive seizure, and then went into a deep coma. This was accompanied by high fever. He apparently had a slight headache for a short period preceding this explosive onset.

On examination about five hours after the onset, the child appeared so ill that it seemed as if a fatal outcome were imminent. He was in a deep stupor and was markedly cyanosed. He had twitchings of the right side of the face and the right arm. There was no rigidity of the neck, Kernig sign or Brudzinski sign. There was a positive Babinski reflex on the right side. All the deep and superficial reflexes were present. There was a nystagmoid movement of the eyes to the left. The pupils were widely dilated and did not react to light. The lungs presented the signs of pulmonary edema. The temperature was 104 F., and the pulse 120; the respirations were Cheyne-Stokes in character.

A lumbar puncture performed shortly after the onset yielded a normal fluid. The following day the child was definitely improved. The temperature dropped to normal and the child regained a certain degree of consciousness. This improvement, however, was of very short duration. Within twenty-four hours the temperature rose and the child lapsed into deep coma again.

May 6, a second lumbar puncture yielded 30 cc. of clear fluid. This showed 15 mononuclears, a slight increase in protein, and a rather high sugar content (84.2 mg. per hundred cubic centimeters). There were no organisms on smear or culture.

Within two or three days the clinical picture changed decidedly. The child's mind appeared blank, and his eyes showed a meaningless and aimless stare. At the same time he was extremely restless and hyperactive. He constantly tossed about and made repeated efforts to scale the crib. He vigorously and violently resisted all attempts to restrain him. He showed definite negativism, and refused to take nourishment. As a result gavage feeding was resorted to. Except for an occasional shrill cry, he was speechless. He had a low, irregular fever. A few days later another complication appeared. He began to have frequent petit mal seizures. This hectic clinical picture persisted for about two weeks. Then the temperature dropped to normal and he began to take food by mouth. The restlessness abated somewhat. The petit mal seizures, however, persisted, though they became less frequent. He began to show some interest in his surroundings, and would follow his mother around. At the same time he began to utter a few words. At this point his progress ceased and for the succeeding few months his condition remained practically unchanged. At the last examination he was in excellent condition physically but seemed to have improved very little mentally. He was still restless and apparently unaware of his surroundings. Some of his memory associations, however, have been restored. He would frequently sing an isolated line of an old favorite song and would repeatedly call out his teacher's name. He would also call for his mother or father occasionally. His speech was thus limited to a few isolated phrases and words, pronounced rather indistinctly. He did not seem to undertand when spoken to, nor did he seem to be able to interpret objects. The petit mal seizures still recurred at varying intervals. At that time it seemed to us that the boy was destined to remain a mental defective.

We have cited this case in detail in order to illustrate some interesting and important points. This was by far the most severe and malignant form of the disease which did not end in death. It was the only case that ran a chronic course. A third point of importance is the apparent extensive injury to the brain as shown by the present clinical picture. Although it is difficult to give a definite and accurate prognosis, it seems as if the mental changes in this case will be permanent.

COMMENT

Of the twelve patients that we have seen with this disease, three died, a mortality of 25 per cent. Seven of the nine who recovered were followed up. All except the one just cited made a complete recovery.

It seems fairly certain that an acute encephalitis is occasionally closely associated with various acute infections, especially measles. Whether or not this encephalitis bears any relation to the epidemic form it is at present impossible to say. It is true that all these cases that we have recorded have occurred since 1918. It is possible that physicians have taken more interest in such conditions on account of the discussion stimulated by the prevalence of epidemic encephalitis. It is obviously impossible to make a definite statement as to the etiology and pathology of this condition. The subject certainly merits further study.

For bibliography see the original article.

MENINGITIS

Distribution According to Age and Etiology

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Josephine B. Neal

In the recent literature a number of interesting papers have appeared dealing with the incidence of meningitis in early infancy, particularly in the first two or three months of life. A study of these papers reveals the fact that such cases are comparatively rare. Three very valuable contributions to this subject are the articles by Barron, in 1918; by Root, in 1921, and by Cooke and Bell, in 1922. These papers contain extensive reviews of the literature and complete bibliographies. No attempt will be made in this paper to review the literature again, but references will be made to the statistics collected therein for purposes of comparison with our own tables.

Since 1910, when the meningitis division of the department of health of New York City was established by Dr. W. H. Park, there has been an opportunity for the members of that division to study a rather large number of cases of meningitis. During that time there has been no epidemic of meningococcic meningitis. Therefore Table 1 represents what may perhaps be considered a normal distribution of meningitis in New York City from an etiologic standpoint. It shows 1,523 cases of meningitis arranged according to age and the more common etiologic agents.

In addition, we have seen the following cases due to more unusual organisms: Friedländer's bacillus, two patients, one aged 2 years and one adult; B. *pyocyaneus,* one patient, aged 7 years; *Micrococcus catarrhalis,* one patient, aged 7 weeks; Streptothrix group, one patient, aged 12 years; torula, one patient, aged 16 years.

The cases due to mixed infections are as follows: meningococcus and staphylococcus, one patient, aged 9 years; meningococcus and B. *paratyphosus* B, one patient, aged 7 months; *Streptococcus hemolyticus and Staphylococcus albus,* one patient, aged 3 months; streptococcus, pneumococcus and staphylococcus, one patient aged 7 years; streptococcus (nonhemolyzing) staphylococcus and unidentified gram-amphophilic coccus, one patient, aged 8 years; a mixed infection containing six organisms, which were never satisfactorily classified, the only identified pathogenic organism belonging to the *B. coli* group, one patient, aged 16 years. This makes a total of 1,535 cases.

TABLE 1.
DISTRIBUTION OF MENINGITIS ACCORDING TO AGE AND ETIOLOGY

Age	Tubercle Bacillus	Meningococcus	Pneumococcus	Streptococcus	Influenza Bacillus	Staphylococcus	Colon Bacillus	Total
3 months	5	24	3	11	2	0	3	48
3 to 6 months	21	58	4	6	8	0	0	97
6 to 12 mos.	78	68	11	3	14	2	0	176
No. cases under 1 year	104	150	18	20	24	2	3	321
1 to 2 years	178	79	8	9	14*	2	1	291
2 to 3 years	80	36	9	6	6	1	0	138
3 to 5 years	99	80	6	11	1	2	0	199
5 to 10 years	96	105	16	20	3	0	1	241
10 to 20 years	57	101	7	5	3	2	0	175
Over 20 years	48	74	22	12	0	2	0	158
Totals	662	625	86	83	51	11	5	1,523

Of this number, fifty were 3 months old or younger. It is interesting to observe that twenty-four of these cases were due to the meningococcus, eleven to the streptococcus, five to the tubercule bacillus, three to the pneumococcus, three to the *Bacillus coli*, two to *Bacillus influenzae*, one to *Micrococcus catarrhalis*, and one to mixed *Streptococcus hemolyticus* and *Staphylococcus albus*. This is in marked contrast to the report of Barron, who compiled from the literature thirty-nine cases of meningitis in infants under 3 months of age, and found that thirteen were due to *Bacillus coli* and only five to the meningococcus. He reported another case also due to *Bacillus coli*. In many of the cases reported in the literature, the diagnosis was made at necropsy and there is a possibility, perhaps, that the *Bacillus coli* isolated may have been an invader just before or just after death, and not the specific cause of the meningitis. Since Barron's article, other cases of meningitis in young infants, due to members of the colon group, have been reported by Greenthal, Smith and Aberd, Sherman, and Cooke and Bell. In view of our own experience, covering a relatively large number of infants under 3 months of age, we must consider *Bacillus coli* as a comparatively rare cause of meningitis in infants.

That the streptococcus is a not uncommon cause of meningitis in infants is shown by the fact that eleven of the fifty cases were due to this organism. Similar findings have already been reported by Holt and Koplik.

The only reference we have been able to find to tuberculous meningitis in early life is by Holt, who reported three cases in infants under 3 months of age in a series of 218 children under 3 years of age.

The number of cases due to the meningococcus, twenty-four, is surprisingly large, compared with other reports. Cooke and Bell reported in 1922, that they were able to find only twelve cases in the literature of the last five years. To these they added four cases, and Root's case also should be added. Root compiled from the literature thirty-four cases of meningitis in infants under 2 months of age: due to the colon bacillus, eleven; to the staphylococcus and streptococcus, nine; to the meningococcus, six; to the pneumococcus, four; to *Bacillus lactis-aerogenes*, one; to *Bacillus mucosus capsulatus*, one; to *Bacillus pyocyaneus*, one, and to *Micrococcus catarrhalis*, one.

For comparison with these figures, we have tabulated our own cases of infants under 2 months of age (Table 2).

TABLE 2.

CASES OF MENINGITIS IN INFANTS UNDER TWO MONTHS OF AGE

	Age and Number	Total
Streptococcus	6 weeks, 3; 7 weeks, 2; 8 weeks, 1	6
Meningococcus	3 weeks, 2; 7 weeks, 1	3
B. coli	2 weeks, 1; 5 weeks, 1	2
B. influenzae	2 months, 2	2
Pneumococcus	2 months, 1	1
Micrococcus catarrhalis	7 weeks, 1	1
Total		15

It will be seen that our youngest case was that of *Bacillus coli* meningitis in an infant, aged 2 weeks. Two cases due to the meningococcus were in infants aged 3 weeks.

It is generally recognized that meningitis is essentially a disease of childhood. It is, perhaps, not generally realized that so large a proportion of cases occur in children during the first year of life. It will be seen in Table 1 that far more cases of meningococcic meningitis are found in children in the first year than in any one year; indeed, that the number in the first year is nearly twice as great as that in the second year, and more even than in the five year group from the fifth to the tenth years. In all other forms of purulent meningitis also, there are more cases in infants in the first year than in any other year. In tuberculous meningitis the number of children in the second year of life exceeds that in the first year; but, taking all forms of meningitis together, the greatest number is found in the first year.

Adding together the cases of patients under 10 years of age, it will be seen that 1,190, or more than 78 per cent. of the total number, occur in this age group.

As regards the etiologic distribution, it will be noted that more cases of tuberculous than of meningococcic meningitis have been seen. As was stated earlier, since 1910 there has been nothing approaching an epidemic of meningococcic meningitis in New York City. Indeed, since the spring of 1919, the number of cases has been extremely small. After the meningococcus, the pneumococcus will be seen to be the most common cause of purulent meningitis. Eighty-six cases were due to this organism. Eighty-five cultures from the spinal fluids of patients who had pneumococcus meningitis have been typed. A part of these were fluids sent in by physicians from patients that we did not see. Most of the patients, however, were seen by us. The results were as follows: Type I, eighteen; Type II, twenty-two; Type III, fifteen; Group IV, thirty.

The number of cases of meningitis due to the streptococcus, eighty-three, almost equals those due to the pneumococcus. *Streptococcus viridans* very rarely causes streptococcic meningitis. Practically all the cases are due to one of the hemolyzing streptococci.

Bacillus influenzae is the fourth most common cause of purulent meningitis, causing fifty-one cases in our list, with the staphylococcus and *Bacillus coli* ranking fifth and sixth, respectively. The number of cases caused by these last two organisms is very small.

Practically any of the pyogenic organisms may occasionally invade the meninges, and set up a meningitis. The same is true of the higher organisms, such as members of the streptothrix group, and the pathogenic yeasts, of each of which we have one case in our list. It will also be seen that various organisms may combine to set up mixed infections, though, in our experience, mixed infections are comparatively rare.

Summary

In a list containing 1,535 cases of meningitis arranged according to age and etiology, it is seen that:

1. With the exception of tuberculous meningitis, more cases of meningitis occur in the first year of life than in any other year. The number of cases of meningococic meningitis in the first year of life far exceeds those in any other year.

2. The greatest number of cases of tuberculous meningitis is found in the second year of life.

3. In times when there is no epidemic, the number of cases of tuberculous meningitis equals or exceeds the number of cases of meningococcic meningitis.

4. After the meningococcus, the pneumococcus and the streptococcus are the most common causes of purulent meningitis, followed by the influenza bacillus, the staphylococcus and *Bacillus coli,* in the order named. Cases due to the last two organisms are comparatively rare.

5. Other pyogenic organisms occasionally cause a meningitis, and, more rarely, members of the higher group of organisms, such as the members of the streptothrix group and the pathogenic yeasts. Mixed infections are rare.

For bibliography see the original article.

EPIDEMIC MENINGITIS

A Study of More Than Six Hundred and Fifty Cases, with Especial Reference to Sequelae

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Josephine B. Neal, Henry W. Jackson and Emanuel Appelbaum

While epidemic meningitis is by no means a rare disease, the number of cases occurring when there is no epidemic is small. It seems worth while, therefore, to call attention to certain facts in regard to the disease based on a study of meningeal conditions in New York covering a period of sixteen years. During this time there has been no epidemic, so that the data show what occurs under normal conditions.

We have been impressed with the fact that most of the laity and many physicians think that patients recovering from epidemic meningitis are liable to be seriously handicapped, especially in their mental development. For this reason we have followed up as many as possible of our patients who recovered to learn what percentage of cases show after-effects, and the nature of them.

Epidemiology

Epidemic meningitis is apparently a disease to which most persons have a natural immunity. In our study we have on record only fourteen instances in which more than one case occurred in the same family. As most of these cases have been in crowded districts, it is obvious that these 654 patients have exposed to the infection several thousand persons. In spite of this we have not found cases occurring in neighboring apartments, except once. In this instance, a man residing in an upper apartment developed epidemic meningitis at about the same time as a case occurred in another family occupying a lower apartment in the same building.

It not infrequently happens that epidemic meningitis is superimposed on some other acute infection, particularly pneumonia or influenza.

In this locality by far the greatest number of cases have occurred during the first six months of the year. Table 1 shows the seasonal distribution.

A study of the distribution by sex of 650 cases shows a preponderance of males, nearly in the ratio of 3:2, as indicated in table 2.

TABLE 1.

SEASONAL DISTRIBUTION OF EPIDEMIC CEREBROSPINAL MENINGITIS

Year	January, February, March	April, May, June	July, August, September	October, November, December	Total
1910	..	..	1	4	5
1911	3	9	6	4	22
1912	8	6	4	7	25
1913	9	10	1	4	24
1914	25	11	5	10	51
1915	14	10	5	6	35
1916	22	28	10	15	75
1917	19	45	9	12	85
1918	51	46	19	20	136
1919	20	19	9	9	57
1920	13	5	4	5	27
1921	15	9	5	7	36
1922	13	8	2	5	28
1923	6	6	3	3	18
1924	3	6	3	1	13
1925	5	1	5	6	17
Total	226	219	91	118	654
Seasonal distribution by percentage	34.6	33.5	13.9	18	

TABLE 2.

SEX DISTRIBUTION OF EPIDEMIC CEREBROSPINAL MENINGITIS

1925 Inclusive

	Male	Female	Total	Unknown
3 months	16	6	22	1
3 to 6 months	37	26	63	..
6 to 12 months	56	24	80	..
1 to 2 years	50	27	77	1
2 to 3 years	15	22	37	..
3 to 5 years	42	39	81	..
5 to 10 years	50	56	106	2
10 to 20 years	57	44	101	..
20 to 30 years	40	14	54	..
30 to 40 years	7	11	18	..
40 to 50 years	3	7	10	..
50 to 60 years	0	1	1	..
Total	377	277	650	4
Percentage	57.5	42.5		

A study of the age distribution reveals some interesting and instructive facts. A glance at table 3 will readily show that epidemic meningitis is largely a disease of infancy and early childhood. A far larger number of cases occur during the first year of life than in any other one year. This, incidentally, raises the mortality rate, as this disease is especially fatal during early infancy.

TABLE 3.

AGE DISTRIBUTION

3 months	23
3 to 6 months	63
6 to 12 months	80
Total under 1 year	166
1 to 2 years	78
2 to 3 years	37
3 to 5 years	81
5 to 10 years	108
10 to 20 years	101
20 to 30 years	54
30 to 40 years	18
40 to 50 years	10
50 to 60 years	1
Total	654

ETIOLOGY

The causative agent of epidemic meningitis is the meningococcus, the presence of which in the spinal fluid must be proved by culture before an absolute diagnosis can be made. It is probable that there is a brief invasion by the organism into the blood in practically all cases of epidemic meningitis. It is now commonly accepted that the organisms usually enter the blood stream through the mucous membrane of the nasopharynx, and then localize in the meninges. While the presence of meningococci in the blood is usually transient, in a certain type of case, the septicemic, they may persist in the blood stream for a considerable period of time, either before or after the meningeal localization. It occasionally happens that the meningococci remain in the blood and do not reach the meninges, thus producing a true meningococcemia.

SYMPTOMATOLOGY

While many writers divide epidemic meningitis into a variety of classes, an adequate conception of the disease may perhaps be expressed by presenting four clinical pictures; that of the most common type occurring in older children and adults; that occurring in infancy; the septicemic type, and the fulminating type. The clinical picture in all is modified by the severity of the infection and the resistance of the individual.

The Usual Picture in Older Children and Adults.—The classic picture of epidemic meningitis in older children and adults is fairly familiar to all. There is a sudden onset, with headache, vomiting and fever of an irregular type. Convulsions may occur, especially in children. Hyperesthesia is fairly common. Delirium is not uncommon. There is a petechial eruption in 12 or 15 per cent. of cases, which soon fades. An herpetic eruption is fairly frequent. The bowels are usually constipated, and there is sometimes retention of urine. Within the first twenty-four to forty-eight hours, stiffness of the neck and the Kernig and Brudzinski signs appear. Early in the disease, the tendon reflexes are likely to be exaggerated and equal. Later they may become unequal, diminished and finally lost. The reaction of the pupils to light follows the same course. In children, a positive Macewen sign indicating increased intraventricular pressure can usually be detected. Strabismus occasionally occurs, more frequently in children than in adults.

The course of the disease in a treated case depends on how early the serum is administered and the response of the patient to treatment.

There are four courses which the disease may pursue: 1. The disease may run an acute course, the patient either dying or recovering after a few doses of serum. 2. The disease may be greatly prolonged, so that twenty or more doses of serum may be given before recovery or death finally takes place. 3. The disease may relapse after apparent recovery, indicating, perhaps, some focal or walled off infection that breaks down. In this type of case recovery may take place. We have comparatively few cases belonging to this class. 4. There is a class in which there is blocking at the base. This type, with few exceptions, is fatal.

The Clinical Picture in Infancy.—It has already been shown in table 3 that more cases occur in the first year of life than in any other one year. It is at this age that the mortality is highest and the diagnosis most difficult.

In infancy we do not see early the stiffness of the neck, Kernig's sign and the changes in reflexes that characterize the disease in older children and adults. The onset is less sudden. There is often vomiting and practically always some intestinal disturbance. There is an irregular fever. The gastro-intestinal condition does not respond to the usual method of treatment. Convulsions often occur early, but these are so common in other diseases of infancy that they are not diagnostic. Usually ten days or two weeks have elapsed before symptoms develop that direct the physician's attention to the central nervous system. These may be the developmnt of strabismus, a fairly well defined rigidity of the neck or severe convulsions. Much earlier, a bulging of the fontanel might have been detected if an examination of the child had been made when not crying. The pupillary and patellar reflexes are often normal when the case is well advanced. Certainly, an irregular fever and a gastro-enteritis that does not respond readily to treatment especially when there is a bulging fontanel, are sufficient indications for an early lumbar puncture. It is in these young children that a walling off at the base with a resulting dry tap most frequently occurs.

The Septicemic Type.—This type of meningitis is quite rare among sporadic cases. In certain of the army camps during the war there was a very large percentage of this type of case. The early picture is that of a septicemia rather than a meningitis, the meningeal symptoms developing five or six days or more after the onset. A profuse and rapidly spreading macular or purpuric rash is an almost constant feature. The rash usually persists for some time. Other indications of a generalized blood infection, such as joint involvements, may be present even before the meningeal symptoms.

The Fulminating Type.—This form is rare. It is characterized by a violent onset. The patient is usually seized with severe headache and vomiting, and speedily loses consciousness. The course is very brief, death supervening within from twenty-four to forty-eight hours. It sometimes happens that the patient dies in an even shorter period. There are cases on record in which the patient retired in apparently good health and was found dead in the morning. The importance of this from a medicolegal standpoint must be borne in mind. It may be of interest to note that this form of meningitis is most likely to occur in persons of the status lymphaticus type.

DIAGNOSIS

There is a large number of diseases which may in some respects simulate epidemic meningitis. The following are the more important conditions that may cause confusion in the diagnosis: other forms of purulent meningitis; occasionally, tuberculous meningitis; acute anterior poliomyelitis (especially the nonparalytic type); epidemic encephalitis; brain abscess; sinus thrombosis; cerebrospinal syphilis; tetanus; meningeal or cerebral hemorrhage; uremia; meningism associated with acute infections, such as gastroenteritis, acidosis, tetany and typhoid; typhus fever; bacterial endocarditis; purpura hemorrhagica; the acute leukemias, and certain forms of drug and metallic poisonings.

Space does not permit a discussion of the differentiated points of diagnosis. An absolute diagnosis of epidemic meningitis depends on the demonstration by culture of the meningococcus in the spinal fluid. A presumptive diagnosis, however, may be made in cases showing the characteristic changes of meningitis in the spinal fluid, and responding to serum treatment, even though the presence of meningococci has not been demonstrated.

LABORATORY OBSERVATIONS

The blood shows a varying increase in the leukocyte count, and there is a polymorphonucleosis. It has been quite definitely proved that there is usually an early transient bacteremia. As has already been stated in the septicemic type of the disease, the meningococci persist in the blood for a comparatively long time.

The examination of the spinal fluid is by far the most useful aid in diagnosis. Netter states that in the first twenty-four hours the fluid is practically almost clear and mononuclears predominate. We have seen very few cases so early as this. In the early stages of the disease the fluid is increased in amount and shows varying degrees of turbidity. The cells are greatly increased and the polymorphonuclears predominate.

The stained smear shows varying numbers of gram-negative diplococci, both intracellular and extracellular. The protein content is moderately to greatly increased. The sugar content may be normal early in the disease, but later it practically always is diminished or absent. With improvement in the clinical picture there is usually a decided rise in the amount of sugar. We regard this as a valuable prognostic point.

On suitable mediums, the meningococci develop in twenty-four hours or more. Their identity may be proved by agglutination with meningococcus serum and the fermentation test. A definite diagnoses cannot be made from the smear alone, as meningitis may be caused by other gram-negative cocci; moreover, the gram-positive cocci are sometimes easily decolorized in the smear.

In one instance, when the diagnosis was not made during the acute stage and serum was not administered, the patient's serum showed well defined complement fixation with the meningococcic antigen. We do not regard this as a valuable diagnostic aid, as it has failed in other cases.

TREATMENT

We have always favored a conservative method in treatment. We ordinarily give serum every twenty-four hours, injecting 20 cc. by gravity intraspinally if as much or more of the fluid has been obtained. In certain instances, when only a small amount of fluid is obtained, we inject more than the amount of fluid withdrawn, provided the serum runs in easily by gravity and no untoward symptoms result. We continue the daily intraspinal injection of serum until the fluid is sterile. After this the injection of serum is determined by the clinical picture. It is often necessary to do one or more lumbar punctures during convalescence for relief of pressure without administering serum. In the average case with recovery, from six to ten doses of serum are required. It is rarely safe to give less than four injections. In very severe cases we often give the second and third injections at eighteen hour intervals. In the septicemic type of case in adults, we administer the serum intravenously as well as intraspinally. We do not, however, approve of the intravenous method of serum administration in children, concurring in this with the opinions of Netter and Blackfan. Incidentally, the septicemic type is rare in children.

During the last few years there has been a trend toward a more intensive method of treatment. Advocates of this method give serum every twelve or even every eight hours intraspinally, and also give it intravenously and intra muscularly in every case. In order to compare the results of this method of treatment with our own, we studied the records of a children's service at an excellent hospital in the city from January, 1918, to October, 1921. During this period, seventy-nine cases were treated at that hospital by the more intensive method, with a mortality of 54 per cent. During the same period we treated 254 cases, with a mortality of 23 per cent. This seemed to us very strong proof that there is no advantage in the more heroic method of treatment. Much attention has been called to the favorable results following the intravenous use of serum in certain of the army camps in which the septicemic type predominated. It is important, therefore, to note that successful results were obtained by the intraspinal method of treatment alone in those camps in which the septicemic type of case did not prevail. In a personal communication, Dr. Harold E. Smith stated that at Camp Mills eighty cases were treated only by intraspinal injections with a mortality of 10 per cent.—an excellent result. It certainly seems to us that the enthusiasm for administering serum intravenously in cases other than the septicemic type is ungrounded.

It quite frequently happens that in infants there is a blocking at the base of the brain, so that little or no fluid can be obtained by lumbar puncture. In these cases it is necessary to resort to ventricular puncture for the withdrawal of fluid and the injection of serum. By this method the ventricular fluid is usually rendered sterile. However, in all but two instances the adhesions have persisted with resulting hydrocephalus, and death has followed. The ventricular puncture is usually performerd daily in alternate ventricles. When adhesions occur along the spine a cisternal puncture has been resorted to for relief of pressure. As we have been afraid of pressure on the medulla, we have not given serum by this method. In young babies the ventricular puncture is safer than the cisternal puncture. Reports have been made to us of hemorrhage following cisternal puncture in infants. In older children and in adults we have resorted to trephine in order to do a ventricular puncture. The results have been uniformly unsatisfactory. It is important to note that failure to obtain fluid by lumbar puncture does not always indicate adhesions, as it sometimes happens in these cases that a considerable amount of serum may be introduced readily by gravity. In a number of instances there has been recovery in cases of this type. A recent article[1] emphasizes the value of both ventricular and cisternal punctures in advanced meningococcus meningitis. As stated, our results have been decidedly unsatisfactory in the number of cases treated by more radical methods. It is to be noted that the author of the article referred to cites very few cases (and only one of his own) on which to base his opinion. We have on record many similarly obstinate cases treated only by spinal puncture in which the results were equally brilliant and successful. However, we appreciate that in rare instances ventricular or cisternal punctures may be valuable adjuvants to the spinal method.

In cases that have become chronic, we have used autogenous vaccine sometimes with apparently satisfactory results, both intraspinally and subcutaneously.

The general treatment is largely symptomatic. The three points that must always be borne in mind are that the patient must be kept quiet by adequate sedatives; he must have sufficient nourishment, and attention must be paid to elimination, as constipation is usually present and retention may occur. The patient should be kept in a quiet, dark room with proper ventilation.

MORTALITY

A certain number of cases were omitted from the mortality statistics chiefly because we were unable to obtain cooperation in carrying out the treatment. Our mortality for 627 cases is nearly 30 per cent. Certain factors have operated to make this mortality so high. The large percentage of children under 1 year of age is an important factor. Most of our patients had been ill several days and some as long as two or three weeks before we were called in consultation. A certain number of patients had pneumonia or some complication not directly connected with the meningitis which was a contributing factor in the cause of death.

In certain years the mortality has been very satisfactory; for example, from July 1, 1916, to July 1, 1917, eighty-five patients were treated, with a mortality of 23.5 per cent.; from July 1, 1917, to July 1, 1918, 112 patients were treated, with a mortality of 19 per cent.; from July 1, 1918, to July 1, 1919, seventy-eight patients received treatment, with a mortality of 20.5 per cent. Table 4 shows the mortality by age groups.

TABLE 4.
MORTALITY BY AGE
1925 Inclusive

	Recovered	Fatal	Total	Mortality, per Cent.
Under 1 year	83	72	155	46.5
1 to 2 years	51	21	72	29.2
2 to 5 years	92	23	115	20
5 to 10 years	87	22	109	20.2
10 to 20 years	74	23	97	23.7
20 to 30 years	35	18	53	34
Over 30 years	18	8	26	30.8
Total	440	187	627	29.8

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[1] Peet, M. M.: Advanced Meningococcus Meningitis, J. A. M. A. 86: 1818 (June 12) 1926.

SEQUELAE

Many of the impressions in regard to the sequelae of epidemic meningitis seem to be based on the history obtained from parents who are prone to ascribe the cause of some defect, especially mental, to a previous illness. In view of the many diseases that may be confused with epidemic meningitis, unless a careful study of the spinal fluid is made, it has seemed to us important to determine the final outcome in a fairly large number of cases definitely diagnosed.

Our study shows that the great majority, 82 per cent., make a complete recovery. About 18 per cent. of the cases do show sequelae, and these are often of a serious nature. It is important to note that the defects for the most part develop during the acute stage of the disease and are the direct and immediate result of the inflammatory process. This is in marked contrast to the sequelæ of epidemic encephalitis which develop months or even years after the more acute process has subsided.

The most important and frequent of the sequelæ is deafness. This is usually bilateral, complete and permanent. In a certain number of cases, however, there is partial or complete recovery from the deafness. In the younger children the deafness was accompanied by mutism. Once there was mutism not associated with deafness. Another patient had halting speech.

Defects of vision are of rarer occurrence. They were found in seven instances. In one case there was bilateral defective vision secondary to hydrocephalus. In the remaining six there was an iridochoroiditis which terminated in a panophthalmitis resulting in complete loss of vision except in one instance, in which, after a short period of complete blindness, the patient regained power to perceive light. It should be noted that the blindness was unilateral in all but one case.

There is a widespread impression that epidemic meningitis is liable to be followed by defective mental development. Our experience does not bear this out. In only eight instances was there any indication of residual disturbances in mentality or intelligence. Four patients, one of whom suffered from hydrocephalus, were definitely subnormal mentally. Three patients complained of nervousness, which may or may not have been the result of the meningitis. One patient became confused occasionally in conversation, but he was able to do clerical work.

Eleven patients showed some form of paralysis, the detailed distribution of which may be noted in the table. These paralytic patients showed a tendency to improve in many instances, and some of them gave promise of complete recovery in the future.

In those patients suffering from headache or general weakness, it was difficult to decide to what extent the meningitis was actually responsible for these conditions.

Rarer but nevertheless important sequelæ were also encountered. In two instances there were sphincteric disturbances—one rectal and one vesical. One patient developed a pachymeningitis nearly two years later. Another patient developed a complete transverse myelitis, which had its inception early in the convalescent period. Table 5 gives a detailed list of the sequelæ.

The total number of cases followed up was 337. In 276 of these, or 81.9 per cent., there was complete recovery, while 61, or 18.1 per cent., showed sequelæ. In several instances there was more than one sequela.

TABLE 5.
SEQUELAE

		Number	Per Cent.
1. Impairment of hearing		26	7.7
(a) Totally deaf	21		
(b) Partially deaf	5		
One was totally blind in one eye			
One had headaches			
2. Paralyses		11	3.2
(a) Four had strabismus			
(b) Right leg and thigh and ankylosis of spine, lumbar region			
(c) Right arm			
(d) Two fingers of right hand			
(e) Right leg			
(f) Strabismus and facial paralysis			
(g) Right hand slightly			
(h) Left arm and leg			
3. Mental disturbances		8	2.3
(r) One hydrocephalic; weakness of legs and ataxic gait; some defect of vision; retarded development			
(b) Two showed poor mental development			
(c) Three, nervousness			
(d) One mentally confused occasionally			
(e) One poor progress in school, forgetfulness and headaches			
4. Defects of vision (5 plus 2 already listed)		7	2.1
(a) One blind in both eyes, nervous, fretful			
(b) Three blind in one eye			
(c) One defective vision in one eye and also nervousness; one case has already been classified under defective hearing, and a second under metal defects			
5. Headaches		3	0.9
6. Speech defects		2	0.6
(a) One had halting speech			
(b) One mute but could hear			
7. Sphincteric disturbances		2	0.6
(a) One rectal			
(b) One difficulty in voiding			
8. General weakness		2	0.6
(a) One unable to work			
(b) One complained of pain and stiffness of back and general weakness			
9. Pachymeningitis nearly two years after illness		1	0.3
10. Transverse myelitis with rectal and vesical involvement and paralysis of both lower extremities		1	0.3

EPIDEMIC MENINGITIS IN THE FIRST THREE MONTHS OF LIFE

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The literature on epidemic meningitis in infancy is meager. Studies of meningitis in early infancy with reviews of the literature have been made by Barron, Root, and Cooke and Bell, and an interesting case of epidemic meningitis has been recently reported by Sturtevant. In view of the undoubted fact that a considerable number of cases are never reported, it does not seem important to make a very careful study of the literature with an attempt to collect the number of cases.

It appears to be a general belief that all forms of meningitis are rare in early infancy. Barron writes, "Although meningitis is a disease quite common in childhood, it is one that is extremely rare during the early months of infancy." Sturtevant states: "Meningococcus meningitis is an extremely rare disease in early infancy." Similar statements are made by Root and by Cooke and Bell. It is our impression, from our own experience, that meningitis occurs in infants more often than is generally believed.

During the sixteen years that we have been studying meningitis and meningeal conditions in New York City, we have had the opportunity of seeing a fairly large number of cases of meningitis of all types in young babies. It seems worth while, therefore, to report our experience. We are limiting our study to those cases occurring during the first three months of life.

During this time we have seen fifty-four cases with the etiologic distribution shown in table 1. A glance at the table shows the great preponderance of cases due to the meningococcus. This group is also the most important from the standpoint of treatment.

TABLE 1.

ETIOLOGIC DISTRIBUTION OF EPIDEMIC MENINGITIS DURING FIRST THREE MONTHS OF LIFE

Meningococcus	Streptococcus	Pneumococcus	Tubercle Bacillus	Colon Bacillus	Influenza Bacillus	Micrococcus Catarrhalis	Total
25	11	7	5	3	2	1	54

Symptomatology

It is not generally recognized that the symptoms of meningitis in infancy are quite different from those in older children and in adults. Since the symptoms of all purulent forms of meningitis are practically the same, we will discuss in detail the symptoms of epidemic meningitis.

While the onset is usually fairly acute, it is rarely so sudden as in later life. Practically always the disease is ushered in with a disturbance of the gastro-intestinal tract. The stools are usually loose and greenish or slimy. Vomiting is by no means as constant a symptom as in older children and adults. It was absent in ten of our twenty-five cases. These gastrointestinal symptoms do not yield to the ordinary methods of treatment. There is practically always an irregular fever. The child is usually irritable and hyperesthetic. There may be convulsions early, but these are not so severe as those that occur later in the disease. Convulsions are not a constant feature. They were present in practically half of our cases in the early stages. The classic signs of meningitis, such as rigidity of neck and the Kernig sign, are usually entirely absent until the case is well advanced. The reflexes at the beginning are normal, as a rule. A bulging fontanel is practically always found. A hemorrhagic rash is very unusual in infants.

On account of the obvious difficulties in diagnosis, we are usually called in consultation when the disease is fairly well advanced. It cannot be too strongly urged that a lumbar puncture should be done early in infants when the diagnosis is indefinite. Netter says: "Think of meningitis and do a lumbar puncture in every infant sick without apparent cause."

Treatment and Course of the Disease

A certain number of cases of epidemic meningitis clear up very readily with the serum treatment. The fatal cases do not often terminate quickly, the course of the disease usually being three weeks or more. It occasionally happens that a patient who has apparently recovered develops hydrocephalus and dies several months after the acute stage has terminated.

In a number of instances in which ventricular puncture was done and the serum administered by that route, the fluid became sterile but the adhesions remained. In only two of this group of cases was ventricular puncture followed by recovery, and it is doubtful whether a real basilar meningitis had developed in these instances. One older child, not in this series, recovered after ventricular puncture. In several instances we have been able to obtain only a small amount of fluid or have had dry taps, and yet serum has been administered by gravity, with complete recovery.

We have followed a very conservative line of treatment. It is our custom to do a daily lumbar puncture, removing as much fluid as possible and injecting serum by gravity. If 20 cc. or more of fluid is obtained, we do not hesitate to inject about 20 cc. of serum, provided it runs in easily and the patient does not show untoward symptoms. We usually inject a smaller amount of serum than we obtain fluid. But if the amount of fluid is very small we may inject somewhat more serum, the quantity depending on the ease with which it runs in and the condition of the patient. It is necessary to exercise more than ordinary care in removing fluid and injecting serum in these young babies. We do not hesitate to perform ventricular or cisternal puncture if there are definite signs of blocking. Certain workers in meningitis have advocated very early ventricular puncture, but the results thus far published have not been such as to encourage us in adopting it.

We continue the injections of serum as long as there are organisms present in the spinal fluid. Even after the fluid becomes sterile, the serum treatment is continued if the clinical conditions seem to indicate it. Lumbar puncture for the relief of pressure—no serum being administered—may be necessary during convalescence.

The general treatment is that of any other acute infection. It is important to disturb the infant as little as possible. It is necessary to keep the child well nourished.

TABLE 2.

DATA ON CASES OF EPIDEMIC MENINGITIS IN INFANCY

Date	Case	Age	Sex*	Intraspinal Injections	Intraventricular Injections	Outcome	Comment	Sequelae
4/ 2/14	1	3 mo.	Not recorded	3	0	Died	Conjunctivitis	
2/ 9/15	2	3 mo.	♀	1	5	Died	Basilar meningitis	
10/28/15	3	3 mo.	♂	3	0	Recovered	....	Deaf
5/19/16†	4	7 wk.	♂	4	0	Died	Parents refused treatment	
6/12/16	5	3 mo.	♂	5	0	Recovered	Macular papular rash	None
7/20/16	6	3 mo.	♂	4	0	Recovered	Arthritis of knee	None
12/24/16	7	3 mo.	♀	5	0	Recovered	....	None
2/ 1/16†	8	2 mo.	♂	2	0	Died	Treatment refused	
5/ 4/17	9	3 mo.	♂	2	1	Died	Basilar meningitis	
5/13/17	10	3 mo	♂	1	1	Recovered	Unable to trace	
6/ 2/17	11	3 mo.	♀	5	0	Died		
8/23/18	12	10 wk.	♂	4	0	Recovered	....	None
3/18/19	13	3 mo.	♂	4	0	Recovered	....	None
4/21/19	14	3 wk.	♂	4	0	Died	Hydrocephalus developed 3 mo. after acute symptoms	
3/ 4/20	15	11 wk.	♀	8	3—	Died	Basilar meningitis	
5/30/21	16	3 wk.	♂	3	17	Died	Basilar meningitis; fluids became sterile	
7/ 5/21	17	3 mo.	♂	0	Several	Died	Basilar meningitis	
11/ 5/21‡	18	3 mo.	♀	0	..	Recovered	....	None
4/20/22	19	3 mo.	♂	8	0	Recovered	....	None
1/ 6/23	20	3 mo.	♂	7	11	Died	Basilar meningitis	
7/23/23	21	3 mo.	♀	Several	Several	Died	Basilar meningitis; left hospital against advice	
1/12/25	22	3 mo.	♂	7	0	Recovered	....	None
4/ 3/26	23	11 wk.	♂	2	0	Died	Hypersensitive to serum	
4/29/26	24	5 wk.	♂	6	0	Recovered	....	None
10/19/26	25	2 mo.	♀	3	2	Recovered	....	None

* In this table, ♂ indicates male and ♀ female.

† Not counted in statistics, as treatment was refused.

‡ One lumbar puncture, no serum; prompt recovery.

MORTALITY

In our group of twenty-five cases, two are not included in the mortality as treatment was refused. Of the remaining twenty-three patients, eleven died, a mortality of 47.8 per cent. This is somewhat higher than our mortality during the first year of life, 46 per cent., and far exceeds our mortality during the second year of life, 29.2 per cent.

REPORT OF CASE

It may be of interest to report in detail the treatment employed in our youngest patient to recover.

D. I., an Italian boy aged 5 weeks, seen April 29, 1926, had become ill ten days before, when the infant was 25 days old. The onset had been sudden with vomiting, severe convulsions, and a temperature of 104 F.

When the baby was examined, the convulsions and vomiting were persisting and the temperature was still 104 F. The fontanel was bulging. The head was retracted. The patellar and pupillary reflexes were normal. Otherwise, the physical examination was negative.

At the first lumbar puncture, 20 cc. of turbid fluid was obtained and 15 cc. of antimeningitis serum administered. April 30, the baby's general condition was improved. Fluid was not obtained on lumbar puncture, but 10 cc. of serum ran in easily by gravity.

May 1, 5 cc. of turbid fluid was obtained, and 10 cc. of serum administered.

May 2, 3 and 4, spinal fluid could not be obtained, but on each occasion 10 cc. of serum ran in easily by gravity.

After the first lumbar puncture the fontanel ceased to bulge and the infant showed progressive improvement. The vomiting and convulsions ceased and the temperature fell to normal, May 4.

The baby has been kept under close observation for eight months, and it has remained well.

This is a case in which many would have resorted to ventricular puncture or cisternal puncture, and would have attributed the recovery to these methods of treatment. This case is by no means an isolated instance of recovery when little or no fluid has been obtained on several occasions, and yet serum has been administered intraspinally with equally good results.

SEQUELAE

The sequelæ were surprisingly few. Of the twelve patients who recovered, one could not be traced. Of the eleven that could be followed up, one is deaf and the other ten have made a complete recovery.

Table 2 gives the more important data in regard to our cases of epidemic meningitis.

For bibliography see the original article.

A CASE OF MENINGITIS CAUSED BY THE MENINGOCOCCUS AND THE PARATYPHOID B. BACILLUS.

Published in The Archives of Pediatrics, 1925, Vol. XLII, pp. 607-609.

Emanuel Appelbaum

The following case, because of its rarity, was deemed worthy of recording in the literature. The patient, an infant boy seven months old, was seen by us for the first time on June 18, 1921. The history of present illness was as follows: Three weeks previously the child was taken suddenly ill with a high fever (105°F.) During the course of the last three weeks the temperature oscillated between 100° and 104°. For the past week the baby had several convulsive seizures and attacks of projectile vomiting. The bowels were constipated. Mentally the child was quite apathetic.

The family and past history were essentially negative.

Physical examination showed the patient to be acutely ill and apathetic. The temperature was 100°; the pulse 120; and the respirations, 20. There was a double internal strabismus. The pupils reacted well to light. The pharynx was slightly congested. The ears were normal. The lungs were clear. The heart, except for the rapid rate of 120, due to the fever, was normal. The abdomen was moderately tympanitic, and the spleen was not felt. There was no cutaneous eruption of any kind, but there was a moderate tâche cérébrâle. The child was not particularly hyperesthetic. The fontanel was not bulging or tense—in fact, there was a depression in this region. There was, however, marked rigidity of the neck with marked retraction of the head. The patellar reflex was normal on the left, but exaggerated on the right side. The abdominal reflexes were present. The Babinski, Oppenheim, Gordon, Schaeffer, and the Chaddock were all negative. No ankle clonus could be elicited. The Kernig sign was positive on both sides, and both the Brudzinski signs were positive. Fundoscopic examination showed a mild optic neuritis in both eyes.

The diagnosis lay between epidemic cerebrospinal and tuberculous meningitis, with many points in favor of the latter.

A lumbar puncture was then performed, and 35 cc. of a cloudy fluid was withdrawn under slightly increased pressure. After the removal of the fluid, 20 c.c. of antimeningococcic serum were introduced. Examination of the fluid showed a great increase in cells, mostly polymorphonuclears, a large amount of albumin and globulin, but no reduction of Fehling's solution. A stained smear showed the presence of Gram-negative diplococci. These organisms were also found on the culture and proved to be meningococci.

June 19:—The fluid obtained by lumbar puncture on this date was distinctly purulent. It showed, however, the presence of a trace of sugar, as there was a slight reduction of Fehling's solution. The meningococci were again found on the smear but not on the culture. Antimeningococcic serum was again given intraspinally.

June 20:—Child seemed a little brighter. Lumbar puncture was performed again, and antimeningococcic serum administered. The fluid was in all respects similar to that of the preceding day.

June 21:—No organisms could be found in the stained smear of the spinal fluid this time, although in all other details this last fluid did not differ from the preceding ones. Antimeningococcic serum was again injected after the removal of the fluid.

June 22:—Lumbar puncture on this day resulted in obtaining 25 c.c. of cloudy fluid which still showed a great increase in cells and contained a large amount of albumin and globulin. The sugar content was, however, definitely increased, as there was a better reduction of Fehling's solution. No organisms were found on smear, but the 24 hour culture yielded a Gram-negative bacillus. A study of the morphological and cultural characteristics, and of the agglutination reaction proved this bacillus to be paratyphoid B.

June 23:—Retraction of the head seemed more pronounced. The spinal fluid was still cloudy, containing many pus cells. The protein and sugar content was the same as the day before. The paratyphoid bacilli were found both on the smear and culture. No meningococci were found. The intraspinal injection of antimeningococcic serum was again repeated.

June 24:—The spinal fluid this time showed only a faint reduction of Fehling's solution, denoting a decided drop in sugar content. Paratyphoid bacilli were present. Antimeningococcic serum was injected intraspinally after removal of the fluid.

June 25:—On this date the spinal fluid was very thick and purulent, contained albumin and globulin in large amount, and did not reduce Fehling's solution. Paratyphoid bacilli were present on smear and culture.

No serum was given this time, but a mixed autogenous vaccine, consisting of both meningococci and paratyphoid B. bacilli, was now administered, intraspinally. A few hours after the vaccine treatment there was a marked reaction, the temperature rising to 105°.

June 26:—By this time the baby's general condition was very bad. A lumbar puncture was done however, and vaccine was again administered intraspinally. The fluid was in all details similar to that of the preceding day.

June 28:—The baby was now stuporous. The temperature was 101°. Only four cc. of purulent fluid were obtained by lumbar puncture this time. Like the preceding fluid, it showed the presence of paratyphoid bacilli. A small amount of vaccine was injected intraspinally.

In spite of the active treatment the child became progressively worse and died on the night of June 28.

Comment.

We have here, then, a case of meningitis caused by two different organisms—first, by the meningococcus and followed immediately by the paratyphoid B bacillus. The former organism was recovered from the first three, and the latter from the last six fluids. We have reason to believe that by the use of the anti-meningococcic serum we probably succeeded in controlling the meningococcic infection, but we failed to check the paratyphoid infection by means of the vaccine. It is also probable that the paratyphoid meningitis was the direct cause of death.

MENINGITIS CAUSED BY BACILLI OF THE COLON GROUP

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Vol. CLXII, pp. 740-748.

JOSEPHINE B. NEAL

MENINGITIS caused by organisms of the colon group is comparatively rare. In analyzing more than 1500 cases of meningitis seen by the Meningitis Division of the Research Laboratory up to 1924, only 5 cases were found due to the Bacillus coli in pure culture. A sixth case was due to a mixed infection, one of which was a member of the colon group. There were 2 cases caused by Friedländer's bacillus.

Barron, in a careful study "Meningitis in the Newborn and in Infancy," found that in 42 cases, 39 of which were under three months of age, there were the following classified according to etiology:

TABLE 1.

Bacillus coli	14
Streptococcus and staphylococcus	10
Meningococcus	5
Pneumococcus	4
Tubercle bacillus	3
Bacillus mucosus capsulatus	1
Bacillus influenzæ	1
Bacillus typhosus	1
Bacillus lactis aërogenes	1
Bacillus pyocyaneus	1
Bacillus catarrhalis (?)	1
	42

All except 1 of these cases were collected from the literature. In the earlier cases the diagnosis was often made at necropsy and it seems probable that in many instances the Bacillus coli may have been an invader just before or just after death and not the specific cause of the meningitis. Certainly this distribution by etiology is in marked contrast to that in 50 cases of our own infants during the first three months of life. In our list the diagnosis was made by the examination of the spinal fluid, and the meningococcus was by far the most frequent cause.

TABLE 2.

Meningococcus	24
Streptococcus	11
Tubercle bacillus	5
Pneumococcus	3
Bacillus coli	3
Bacillus influenzæ	2
Micrococcus catarrhalis	1
Streptococcus hemolyticus and Staphylococcus albus	1
	50

The following table shows the cases collected from the literature and also our own cases:

TABLE 3.

CASES OF MENINGITIS CAUSED BY BACILLI OF COLON GROUP

Source.	Year.	Organisms.	Number cases.	Remarks.
Lloyd	1898	Bacillus coli	1	
Concetti	1900	Bacillus coli	4	
Nobecourt and Du Pasquer	1902	Bacillus coli	1	
Kolle and Wassermann	1903	Bacillus coli	13	
Braillon and Merle	1914	Bacillus coli	1	
Milner	1915	Bacillus coli	1	
Michael	1916	Bacillus coli	1	
Frank	1916	Bacillus coli	1	
Barron	1918	Bacillus coli	11	3 additional cases in Kolle and Wassermann.
Sickenga and Munk	1924	Bacillus coli	1	
Meningitis Division	1925	Bacillus coli	6	1 mixed infection.
Total Bacillus coli			42	
Guinon and Simons	1910	Friedländer's bacillus	1	
Bonhoff and Esch	1912	Friedländer's bacillus	1	
Kolle and Wassermann	1913	Friedländer's bacillus	10	
Haig	1919	Friedländer's bacillus	2	
Elias	1924	Friedländer's bacillus	2	
Menetrier and Bertrand-Fontaine	1924	Friedländer's bacillus	1	
Lion and Minvielli	1924	Friedländer's bacillus	1	
Meningitis division	1925	Friedländer's bacillus	2	
Total Friedländer bacillus			20	
Beitzke	1904	Bacillus lactici aërogenes	1	
Kolle and Wassermann	1913	Bacillus lactici aërogenes	2	
Total B. lactici aërogenes			3	
Greenthal	1921	Bacillus acidi lactici	1	
Sherman	1922	Bacillus acidi lactici	1	
Ray	1923	Bacillus acidi lactici	1	
Total B. acidi lactici			3	
Total colon group			68	

As the typhoid group is so closely allied to the colon group it may be mentioned that we have had only one case in which any member of the typhoid group was involved. This was originally a case of meningococcus meningitis but the paratyphoid Bacillus appeared in the fluid during the course of the illness. The case was reported by Appelbaum. A very complete study of typhoid meningitis was made by Bayne-Jones in 1917.

The following table gives certain data in regard to our cases:

TABLE 4.

Year	Case No.	Age	Sex.	Primary focus.	Approximate duration.	Outcome.
				Friedlander	s' Bacillus	
1915	38	2½ yrs.	M.	Pneumonia and otitis media	About two weeks with meningitis	Recovery
1920	221	59 yrs.	M.	Otitis media	Six days	Died.
				Bacillus	Coli.	
1923	29	2 wks.	M.	Not determined	Twelve days	Died.
1920	360	5 wks.	M.	Not determined	About 8 weeks	Recovered from meningitis developed hydrocephalus died almost a year later.
1923	151	8 mos.	F.	Pyelitis	About 3 weeks	Died.
1916	665	1½ yrs.	M.	Not determined	About 4 weeks	Died.
1919	316	6 yrs.	F.	Infection of orbit	Eight days	Died.

As these cases are comparatively rare a brief description may be of interest.

Case Reports. Case XXXVII.—W. S., a boy aged two years and seven months, was admitted to Willard Parker Hospital March 20, 1915. The onset of the illness on March 19 had been sudden, with a fever of 104° F. He had quickly become stuporous. When examined on March 20 the temperature was 104° F., pulse, 160; respiration, 40. There was a slight stiffness of the neck, a positive Macewen sign, and Kernig sign, no Brudzinski or contralateral reflex. The patellar reflexes were exaggerated, the eyes were normal; there was a discharge from both ears and tenderness around the right mastoid. He was somewhat stuporous. On lumbar puncture 50 cc. of turbid fluid was withdrawn and 25 cc. of antistreptococcus serum was injected, as the meningitis was apparently secondary to the ear involvement. The fluid showed a very great increase in cells, 95 per cent. polymorphonuclears, a moderate increase in the albumin and globulin and a normal sugar content. No definite organisms were found in the smear and it was thought that the culture was contaminated. On March 21, 50 cc. of blood-stained fluid was withdrawn and 20 cc. antistreptococcus serum injected. This fluid showed much the same picture as the first, but a Gram-negative diplobacillus (Friedländer's) was cultured from the fluid, also a diphtheroid bacillus, thought to be a contamination. The patient's general condition was slightly better, but at this time ptosis of the left eyelid appeared, together with dilatation of the left pupil and paralysis of the third nerve on the left side. On March 22, 50 cc. of blood-tinged fluid was obtained. This fluid was sterile as were also the fluids withdrawn on March 22, 24, 25 and 27. These late fluids showed a progressive decrease in the number of cells and in the protein content. The patient's condition also improved. On March 24 an autogenous vaccine was given for the first time in a dose of 250 million. As the spinal fluid had already become sterile, the vaccine cannot be given the credit for the cure of the meningitis. During convalescence from the meningitis a pneumonia with a positive blood culture developed, and an operation on the mastoid was performed. The patient eventually made a complete recovery.

Case CCXXI.—Father S., an Italian priest aged fifty-four years, was seen on June 12, 1920. He had suffered from diabetes and nephritis for several years and from a slight right facial paralysis for some time. Three months before, an acute otitis media had developed. He had been taken ill very suddenly on June 7, with vomiting and chilly sensations. He became unconscious within two hours. There had been no fever for the first two days of his illness. When examined on June 12 he had a temperature of 102° F., pulse, 78; respiration, 46. There was moderate stiffness of the neck and Kernig sign.

The pupils were equal and reacted to light. The patellar reflexes were absent. At times he was delirious and at times stuporous. On lumbar puncture 50 cc. of cloudy yellow fluid was obtained and 20 cc. of antimeningococcus serum injected. The fluid showed an enormous increase in cells, practically all polymorphonuclears, a great increase in the albumin and globulin, and no sugar. In the smear there were numerous short Gram-negative diplobacilli both intracellular and extracellular. A pure culture of Friedländer's bacillus was grown. The patient died on June 13.

Of the cases due to the colon bacillus the second was of unusual interest and will be described last in more detail. The others briefly were as follows:

Case XXIX.—Baby S., aged two weeks, was seen January 30, 1923. The baby had vomited at times since birth and there had been greenish stools. It was difficult to tell when the meningitis had first appeared as meningeal symptoms in infancy are so indefinite. The physician in charge of the case thought it had lasted for about a week. When examined on January 30, the temperature was 100° F., the pulse rapid but regular. The fontanel was bulging, the pupils were equal and reacted to light. The other reflexes were difficult to obtain. As in all infants in the earlier stages of meningitis the stiffness of the neck and Kernig sign were absent. On lumbar puncture 30 cc. of cloudy fluid was obtained and 15 cc. of antimeningococcus serum was injected. The fluid showed an increase in cells, practically all polymorphonuclears, a great increase in albumin and globulin, and an absence of sugar. In the smear there were many short Gram-negative bacilli. A pure culture of colon bacillus was grown. These findings were practically the same in the subsequent fluids. On January 31, 5 cc. of blood-tinged fluid was obtained by lumbar puncture and on February second 0.5 cc. of very purulent fluid. On February 3 and 4 ventricular punctures were done, yielding 45 cc. and 10 cc. respectively of purulent fluid. On February 15, the patient's condition was so serious that no puncture was done. Death occurred on the afternoon of February 15.

Case CLI.—B. T., female infant, aged eight months, was seen on April 23, 1923. The onset of the illness was five days before with fever of 104° F., vomiting, convulsions, cough and irritability. There was a serous discharge from the right ear. There was no stiffness of the neck. The Kernig and Brudzinski signs were negative. The fontanel was not bulging. The pupils were equal and reacted to light. The patellar reflexes were normal. There was marked congestion of the pharynx. The heart and lungs were normal. The spleen was slightly enlarged. The temperature was 101° F. pulse, 120; respiration, 34. As the meningeal symptoms were indefinite no puncture was done. On April 24, as the condition was not improving, a lumbar puncture was performed, 15 cc. of clear fluid being withdrawn. This fluid showed a moderate increase in cells, 90 per cent. mononuclears. The protein and sugar were normal and there were no organisms by smear or culture. It was thought that the condition was one of meningism, probably secondary to the otitis media. The child's condition did not improve and a second puncture was done April 27, yielding 30 cc. of slightly blood-tinged fluid. This fluid was apparently normal except for the blood present. On May 5, a third puncture was done, 20 cc. of clear fluid being withdrawn. This fluid was normal except for a very slight increase in mononuclears and in protein. A week later the child seemed worse and showed more evidence of meningeal involvements. On lumbar puncture May twelfth, 3 cc. of very purulent fluid were withdrawn. This fluid showed a very great increase in cells —polymorphonuclears—and in protein and no sugar. Numerous short Gram-negative bacilli were found in the smear and a pure culture was grown of motile, Gram-negative bacilli which fermented, with the formation of acid and gas, dextrose, mannite, maltose, lactose, dulcite and saccharose. This identified the organism as Bacillus coli communior. Later the patient was catheterized and a pure culture of the same organism was isolated from the urine. We, therefore, concluded that the meningitis was secondary to the pyelitis. Lumbar punctures were done daily until May 18 when it was necessary to do a ventricular puncture as but a few drops of fluid could be obtained from the spine. The ventricular fluid was sterile. On May 19, no fluid could be obtained from the spine but the general condition was somewhat better. On May 21 the child was somewhat more restless and the temperature was 103.5° F. A second ventricular puncture was done yielding 20 cc. of blood-tinged fluid which was sterile. On May 24 a little thick blood-tinged fluid was obtained from the spine. This was also sterile. A catheterized specimen of urine was much less cloudy. The temperature was normal and the general condition seemed much better. This improvement continued until the night of May 26 when convulsions began. A ventricular puncture on May 27 yielded 35 cc. of purulent fluid containing large numbers of organisms. The child died on May 28.

Case DCLXV.—E. D., a male infant aged one and a half years, was first seen on October 22, 1916. There had been a sudden onset, with high fever on October 8. There had been no vomiting. On examination the child was stuporous; the neck was stiff and there was a positive Kernig sign. The Macewen sign was very marked. The patellar reflexes were normal. The temperature was 104° F. On lumbar puncture 40 cc. of turbid fluid was withdrawn and 20 cc. antimeningococcic serum given. This fluid showed a great increase in cells—polymorphonuclears—and in protein and a very low sugar content. In the smear a few Gram-negative "cocci" were seen but the culture was sterile. A second puncture was done on October 23, twenty cc. of fluid being withdrawn and 20 cc. of serum being given. The findings in the second fluid were identical with those in the first. The parents refused further treatment and the case was removed to a hospital where the serum treatment was continued. It was seen by us at the hospital on November 17, when 5 cc. of purulent fluid was obtained by lumbar puncture. This showed numerous short, thick, Gram-negative bacilli in the smear and a pure culture of the colon bacillus. The child died a few days later. It is impossible to say whether this was originally a meningococcus infection or whether the cocci seen in the first two smears were very short bacilli. Organisms in spinal fluid often show pleomorphic forms and we, therefore, feel that we can rely very little on morphology.

Of course, the failure of the organism to grow would be more in favor of the meningococcus, as the colon bacillus grows far more readily.

Case CCCXVI.—M. K., a girl aged six years, was first seen December 10, 1919. She had had measles in May. Following this an abscess had developed behind the right eye necessitating the enucleation of the eyeball. She had complained of pains in the right ear for about a month and had seemed tired and irritable for several days. There had been vomiting for four days. She had been worse on the night of December 9, with fever. When examined she was somewhat irritable, there was moderate stiffness of the neck and a marked Kernig and Macewen sign. The pupil was dilated but reacted to light. The patellar reflexes were absent. There was some tenderness over the right mastoid. The eye socket showed some inflammation. There was profuse sweating. The temperature was 102° F., pulse, 120; respiration, 34. On lumbar puncture 40 cc. of cloudy fluid was withdrawn and 20 cc. of antimeningococcic serum administered. This fluid showed a great increase in cells—polymorphonuclears—and in protein and no sugar. The smear was apparently negative but the culture showed a few colonies of Bacillus coli, which were considered a contamination. Lumbar punctures were done and serum was administered on December 11, 12 and 13. The child's condition improved. The spinal fluids were sterile, became clearer, showed a diminution in the protein and an increase in the sugar. On December 14 and 15 the general condition was not so good and the temperature rose. On December 16 the temperature was 105° F. and the pulse, 160. A lumbar puncture yielded 50 cc. of very purulent fluid from which the colon bacillus was again isolated, this time in great numbers. It seemed probable that a nonspecific action of the serum had produced a temporary control of the infection and an amelioration of the condition. I have seen this same occurrence in other cases of purulent meningitis. The child died on December 17.

Case L. S.—By far the most interesting case in the series was that of L.S. He was born on October 6, 1920. The delivery was normal but he showed signs of collapse and difficulty in breathing for about ten hours. He had slight twitchings for the first few days but these ceased and he seemed normal except for signs of a patent foramen ovale, until the onset of his illness, November 2, 1920. His weight at that time was 10 pounds 7½ ounces. He had weighed 10 pounds 3 ounces at birth. The early symptoms were rather indefinite—sight twitchings, vomiting, a temperature ranging from subnormal to 101.5° F. Early on the morning of November 7, he had a severe convulsion and was seen in the evening of that day. At that time he was in a stuporous condition, the head was thrown back, the fontanel was bulging, the patellar reflexes were not elicited. He was taking nourishment badly and was vomiting profusely. The temperature was 101° F., pulse 160 and poor in quality. A lumbar puncture had been done by another physician earlier in the day and 5 cc. of purulent bloody fluid had been obtained. He had reported that meningococci were present in the smear. A second lumbar puncture was done yielding 3 cc. of purulent, sanguinous fluid and as there were marked pressure symptoms a ventricular puncture was done. 45 cc. of very purulent sanguineous fluid withdraw and 20 cc. antimeningococcic serum injected by gravity. This fluid was uniformly sanguinous and was the most purulent that we have ever received at the laboratory where over 12,000 fluids have been examined. When a test tube full was allowed to stand only a thin layer of clear fluid appeared at the top; over nine-tenths was thick pus. The child's condition was so precarious that it seemed doubtful if it would live until morning. The examination of the fluid showed an enormous increase in polymorphonuclear cells and in protein and an absence of sugar. In the smear there were numerous short, thick, Gram-negative bacilli which grew in pure culture. These organisms fermented lactose and dextrose with production of gas and formed indol, thus proving that it was Bacillus coli communis. On November 8 the condition was slightly improved. The convulsions were less frequent and nourishment was being taken better and retained. On ventricular puncture about 40 cc. of fluid similar to the first was withdrawn and 20 cc. of antimeningococcic serum again given as the organism had not yet been determined. On November 9, the baby was having slight convulsive seizures and the pulse was of a poor quality. A ventricular puncture was done, removing 40 cc. of similar fluid, and 20 cc. normal saline was injected into the ventricle, as it seemed safer to do so since so much fluid had been withdrawn. Ventricular punctures were done daily, with one or two exceptions, until November 28, after which a puncture every other day seemed sufficient. Beginning November 18, several doses of autogenous vaccine were given in the ventricle with the saline, as the fluid showed no signs of clearing and vaccine treatment is often beneficial in Bacillus coli infections. The baby's general condition was gradually improving. On November 12, it cried for the first time. The vomiting was less frequent and the feedings were taken better. The pulse became better in quality and somewhat slower in rate. The temperature was usually between 99° and 100° F. The fluid, however, remained under greatly increased pressure and very purulent, although it became less sanguinous. The amount withdrawn varied from 30 to 60 cc. The ventricles were usually injected with saline at each puncture. The findings in the fluid were the same as at the first puncture, except that the growth of the organism became less profuse. Finally the twenty-fifth fluid, withdrawn December 9, was sterile, as were all the fluids thereafter. On December 11, the fluid was conspicuously clearer and showed the presence of sugar for the first time. On December 15, a lumbar puncture was done and 8 cc. of clear fluid obtained. Another lumbar puncture was done on December 18. On December 20, as there were facial twitchings (these had been present earlier but had been absent for over a week) and vomiting, both ventricles were punctures to make sure that there was no intracranial pressure. From the right ventricles 4 cc. of clear fluid was removed and from the left 1 cc. Lumbar punctures were done also on December 23, 26, 29 and January 5.

The temperature was normal after November 27 and the pulse was usually about 120 and of good quality. The general condition improved very satisfactorily. On December 3 the baby's weight was 9 pounds. He gained steadily thereafter until it reached 12 pounds 6 ounces on February 6. The baby slept well, was playful and intelligent, and seemed entirely normal. The head was slightly enlarged, as was to be expected after the prolonged period of increased pressure. At times the fontanel seemed somewhat tense but this lasted only for a short time and did not occur frequently. Finally, however, about March 10 the head began to enlarge rapidly. On March 13, a lumbar puncture was unsuccessful and 60 c.c. of clear fluid was withdrawn from the ventricle. This fluid was normal except for a slight increase in protein. The relief afforded by the puncture was only temporary. Another ventricular puncture was done March 19 and 60 cc. of clear fluid again removed. A suggestion that the baby be taken to a hospital to try operative measures was not acted upon. The hydrocephalus continued and the baby died about 9 months later.

Children under six months of age, suffering from meningitis are particularly prone to develop a subacute arachnoiditis. This is what probably happened in this case—the inflammation continuing and finally completely cutting off the foramen of Magendie. I have seen a similar occurrence in cases of epidemic meningitis when, after ventricular punctures and the intraventricular administration of serum have rendered the ventricular fluid sterile, one or two lumbar punctures have been successful. But in these cases the complete blocking has taken place in a few days, and the duration of the hydrocephalus has been short, death occurring in at most a few weeks.

Conclusions. 1. Meningitis due to members of the colon group is comparatively rare. In studying over 1500 cases of meningitis only 7 were found due to these organisms. In an eighth case there were several organisms present, one of which belonged to this group.

2. Meningitis in the first three months of life is most often caused by the meningococcus—24 out of 50 cases being due to this organism and only 3 to the Bacillus coli.

For bibliography see the original article.

DIRECT CULTIVATION OF TUBERCLE BACILLI FROM SPINAL FLUIDS. OCCURRENCE OF HUMAN AND BOVINE TYPES

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Ruth Gosling and John Montanus

For some years the Meningitis Division of the Department of Health, New York City, has been interested in determining the relative incidence of human and bovine infection in cases of tuberculous meningitis.

In the past we used guinea-pigs as a means of isolating the organism. The technique was as follows: 5 to 8 cc. of spinal fluid or the emulsified sediment obtained by centrifuging 10 to 15 cc. of fluid was injected subcutaneously into the groin of two guinea-pigs. After four or five weeks, or when definite lesions had developed, the animals were chloroformed, autopsied, and the infected glands removed under aseptic conditions and placed in sterile petri dishes. They were then minced with sterile instruments and a large loopful carefully rubbed over the surfaces of three Dorset's and three Lubenau's egg media slants. The tubes were then sealed with sterile paraffin and incubated at thirty-seven and one-half degrees (37.5°) C. When a good growth had appeared, in about twenty-one to twenty-eight days, they were submitted for typing.

Owing to the fact that the guinea-pigs, because of accidental infections, often died before definite lesions could be demonstrated, it occurred to us that, although spinal fluid contains very few organisms, it might be possible to cultivate these organisms by direct planting upon artificial media.

In this paper we are presenting, first, data to show that tubercle bacilli can be cultivated directly; second, that cultivation is possible even when the number of tubercle bacilli present is so small that they cannot be demonstrated in the direct smear of the centrifuged sediment; and third, a new series of cases to show the relative incidence of human and bovine types in tuberculous meningitis.

The sediments from thirty-four fluids were studied. At first Petroff's beef-bouillon egg medium was used. This contains gentian violet as an inhibiting agent for possible contaminating organisms, but since spinal fluids are seldom contaminated when withdrawn under proper aseptic conditions and collected in sterile containers, we decided to use the same media as we used for our glands, namely, Dorset's and Lubenau's egg media. The technique was as follows: As much spinal fluid as remained (including the fibrin web when possible) after the necessary 15 cc. had been taken to make the diagnostic smear, was certifuged at high speed, in the ordinary conical centrifuge tube, for two hours. All but 0.5 cc. to 1 cc. of the supernatant fluid was poured off and discarded. The sediment was resuspended in the remaining fluid by thorough agitation and scraping of the bottom of the tube with a rather stiff sterile platinum loop. This suspension was removed from the tube with a sterile 1 cc. pipette and distributed over the surface of five slants, two Dorset egg, two Lubenau's and one coagulated blood agar slant. The egg media tubes were sealed with sterile paraffin and incubated at 37.5° C. The coagulated blood agar slant served as a control for contaminating organisms and was also incubated at 37.5° C.

All of our plantings from which the tubercle bacillus grew showed good growth in from twenty-one to twenty-eight days of incubation. These cultures were then submitted for typing to Doctor Grund.

Although rabbit virulence is generally conceded to be the final criterion for determination of types, Park and Krumwiede, with whom Dr. Grund was a collaborator, state in their monograph of 1910, the following: "Culturally one can differentiate practically all viruses with surety. In cases of doubt or lack of sufficient experience in the handling of cultures the intravenous inoculation of 0.01 milligrams will settle the question. Observations of culture and virulence must be done in the early generations." All of our typing was done from the first generation.

Two of the cultures in which there was some doubt as to type were injected into rabbits. These, indicated in the following table, proved to be human. The table gives the results of our thirty-four fluids.

It will be noted that, of the thirty-four sediments planted, twenty-five or 73.3 per cent. yielded positive cultures; six remained sterile, perhaps because of the necessity of using the greater amount of available material for the diagnostic smear, and three showed contaminations in both the egg media and the control shortly after planting. Of the twenty-five which yielded growth of tubercle bacilli, two were from sediments which, after prolonged search, failed to show acid fast organisms in smear, and two showed acid fast granules (Granules of Much).

Only one of these thirty-four stains proved to be bovine.

When we first began planting our fluids directly upon egg media, we thought it of interest, whenever the amount of fluid permitted, to run guinea-pig isolations parallel with the direct method. This was carried out with twelve fluids. Two pigs were used for each fluid. Four sets of pigs died within a week to ten days of inoculation and showed no lesions. The plantings from the sediments from these same fluids yielded tubercle bacilli. This experience suggests that if the amount of fluid permits, the direct cultural method, in addition to the animal inoculation, may be the means of confirming the diagnosis and also the saving for further study of a strain which otherwise might have been lost if animal inoculation alone had been relied upon.

We compared the results obtained by our direct method with those obtained earlier by animal inoculation. A series of thirty-four consecutive fluids was taken, some showing positive smears and some apparently negative and we found that the percentage of positive results was the same for both methods.

The point may be brought up that in the direct cultural method there is the danger of the loss of some particular strain, because of faulty technique or of contamination of the media by some organism in the media itself which does not appear until after prolonged incubation. If guinea-pig glands are used, there is the same danger inasmuch as the same medium is used for the primary isolation of the organism from the gland as from the spinal fluid. Of course if the spinal fluid is contaminated, inoculation into the guinea pig will often result in glands from which the tubercle bacillus may be grown in pure culture, but it has been our experience that spinal fluids withdrawn under proper precautions are rarely contaminated. Possibly the question of time may be one to be considered. We were able by direct method to type

TABLE 1.

RESULTS OF DIRECT INOCULATION OF THIRTY-FOUR SPINAL FLUID SEDIMENTS ON CULTURE MEDIA

	Spinal fluid number	Age of patient	Acid fast organisms in smear	Growth on glycerine egg medium	Growth on water egg medium	Type	Slant control
1	X	Child	+	(Petroff)+	–	Human	–
2	Y	Child	+	(Petroff)+	–	Human	–
3	9128	3 yrs.	+	–	(Petroff)+	Bovine	–
4	9324	8 yrs.	+	+	–	Human	–
5	9337	4 yrs.	+	+	–	Human	–
6	9341	5 yrs.	Acid fast granules	conta	minat	ed	Cont.
7	9361	11 mos.	–	+	–	Human	–
8	9362	27 yrs.	+	+	–	Human	–
9	9370	Child	+	+	–	Human	–
10	9376	Child	+	+	–	Human	–
11	9377	Child	+	+	–	Human	–
12	9380	Unknown	+	+	slight growth	Human	–
13	9381	5 yrs.	+	+	–	Human	–
14	9402	3½ yrs.	+	+	–	Human	–
15	9405	2 yrs.	+	+	slight growth	Human	–
16	9412	18 mos.	+	–	–	–	–
17	9414	3 yrs.	+	–	–	–	–
18	9425	2 yrs.	+	+	slight growth	Human	–
19	9434	3 yrs.	+	+	slight growth	Human	–
20	9435	11 mos.	+	+	slight growth	Human	–
21	9436	23 yrs.	–	+	slight growth	Human	–
22	9448	22 mos.	+	–	–	–	–
23	9460	26 yrs.	Acid fast granules	–	–	–	–
24	Z	Child	+	+	–	Human	–
25	9467	14 mos.	+	+	–	Human	–
26	9470	10 mos.	+	+	–	Human	–
27	9590	2 yrs.	+	contaminated		–	–
28	9618	20 yrs.	+	–	–	–	–
29	9563	5 yrs.	+	–	–	–	–
30	9654	1¼ yrs.	+	contaminated		–	B. subtilis
31	9688	9 yrs.	+	–	slight growth	Human	–
32	9704	10 mos.	+	–	slight growth	Human	–
33	9156	5½ yrs.	+	(Petroff)+	–	Human	–
34	9164	Unknown	+	(Petroff)+	–	Human	–

Results:—Human 24 Bovine 1

cultures in twenty-eight days at the most, whereas if guinea-pigs had been used, at least eight weeks would have been necessary for the typing.

As we have indicated in the beginning of this paper, the work of typing was first carried on by guinea-pig inoculation. We isolated by this method twenty-three strains. The results are shown in Table II.

TABLE II.

TYPES OF TUBERCLE BACILLI FOUND IN SPINAL FLUIDS BY GUINEA-PIG INOCULATION

	Fluid Number	Age of Patient	Type
1	7650	3 years	Human
2	7882	8 years	Human
3	7945	2½ years	Human
4	7962	Unknown	Human
5	8136	14 years	Human
6	8200	4 months	Human
7	8310	5 years	Human
8	8318	18 years	Human
9	8477	4 years	Human
10	8499	5 years	Human
11	8503	2 years	Bovine
12	8528	8 years	Bovine
13	8617	8 years	Human
14	8622	Unknown	Human
15	8656	41 years	Human
16	8604	9 years	Human
17	8677	8 months	Human
18	8851	1 year	Human
19	8880	3½ years	Human
20	9007	3 years	Human
21	9118	1 year	Human
22	9305	10 months	Human
23	White	Unknown	Human

Results	Human	Bovine
	21	2

Two of the above twenty-three strains isolated from the guinea pig were bovine. One of the twenty-five isolated by the direct method was bovine. Both of the cases from which the bovine type was isolated by animal passage

TABLE III.

SUMMARY OF CASES OF TUBERCULOUS MENINGITIS CLASSIFIED AS TO TYPE

Reference	Human	Bovine	Total	Percentage of human types
Park & Krumwiede......	123	16	139	88.5
Novick	45	3	48	93.7
Gordon & Brown	4	0	4	100
Gosling & Montanus	45	3	48	93.7
Total	217	22	239	90.8%

were residents of New York City. In one case, 9128, only a very indefinite history of the feeding could be obtained from the mother. The child was a breast-fed infant and later given Grade A milk. It could not be ascertained whether or not the milk was Grade A Raw or Pasteurized. The child at the time of the meningitis was on a general diet.

Case 8503 was breast fed until six months. It was then given Grade B Pasteurized milk which the mother said she "pasteurized" again but did not boil. It was never given what the mother called raw "store" milk.

Case 8528, isolated by direct culture, was an out-of-town case, and no history of the feeding could be obtained.

Thus out of a total of forty-eight viruses, three or 6.3 per cent. were of the bovine and forty-five or 93.7 per cent. were of human origin.

Table III is a summary of the cases in the literature as to the relative incidence of the human and bovine types in tuberculous meningitis. This table includes our own findings.

SUMMARY

We have shown that in a fair percentage of spinal fluids from cases of tuberculous meningitis, in which the organisms were demonstrated microscopically, it is possible to grow the tubercle bacillus in pure culture by direct planting of the sediment upon suitable egg media.

The fact suggests itself that direct planting of the sediment from cases of suspected tuberculous meningitis, where no organisms can be shown in smear, may have some value as a diagnostic procedure and further, that when guinea-pigs are used for the isolation of strains of the tubercle bacillus from spinal fluids, especially when conditions are adverse to the proper care of the animals, due to overcrowding and incidence of epizoötic diseases during the winter months, the planting of some of the sediment directly upon egg media may be the means of saving some strains for further study.

In our series of forty-eight strains of tubercle bacilli isolated from spinal fluids from cases of tuberculous meningitis, three or 6.3 per cent. proved to be of bovine origin.

For bibliography see the original article.

STREPTOTHRIX MENINGITIS

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TEN EYCK ELMENDORF AND JOSEPHINE B. NEAL

The classification of the higher bacteria to which the streptothrix group belongs presents many difficulties, and no attempt will be made in this paper to undertake a discussion of them.

The infections in man reported as being due to the streptothrix group are comparatively rare. The lung is apparently the chief site of infection but metastatic abscesses of the brain and cord are occasionally reported. Cases of true meningitis not secondary to brain abscesses are apparently much less common.

In a study of streptothrix meningitis published by Neuman and Rabinowitsch-Kempner, in 1922, it is stated that they could collect from the literature but three well established cases of streptothrix meningitis. However, they accepted only those cases that were confirmed by autopsy. The cases they report are as follows: 1. A case reported by Sternberg in 1910, a twelve-year old boy. The meningitis was secondary to a very chronic otitis media. 2. A case reported by Weisner in discussing Sternberg's case. The meningitis was secondary to an acute otitis media. 3. A case reported by von Eicken and Huntemüller, in 1919—an 18-year-old boy, the meningitis being secondary to a chronic otitis media.

Their own case was that of a child three years of age. This meningitis also was secondary to an otitis media. The duration of the illness was only three days. Two lumbar punctures were done. The spinal fluid was purulent and showed the streptothrix in pure culture.

They also refer to a case described by Naunyn, in 1891, in which there was a streptothrix pyemia in the course of which a streptothrix meningitis developed.

A study of literature, however, reveals other cases that apparently must be included in the number of cases of streptothrix meningitis.

Horn, in 1913, reported a case in a man of thirty-five who had suffered for many years from otitis media. He developed symptoms of meningitis and also ocular symptoms indicating sinus infection. It is stated that the spinal fluid was negative. The autopsy revealed purulent meningitis, sinus thrombosis and otitis media. The streptothrix was isolated from the purulent material.

In the Italian literature there are reports of 10 cases of streptothrix meningitis, the diagnosis usually being made by cultures from the spinal fluid. The first case was reported by Rutelli in 1915,—a child six months old. Three lumbar punctures were done. The outcome of the case is unknown as the child was taken to the hospital. In 1916, Rudoni reported seven cases of streptothrix meningitis that developed over a short period during a small epidemic of meningococcic meningitis. In five instances meningococci were found earlier in the spinal fluid and the streptothrix appeared later. In two cases the streptothrix was the only organism found. All the cases resulted fatally. This unusually large number of cases of a rare infection occurring within a period of two months makes one wonder what the source of infection could have been. Such conjectures are usually futile, however, and it seems best to refrain from making them. In 1918

Kharina-Marinucci reported a fatal case in a child four months old. In 1922 Fonzo reported a fatal case in a child 12 months old. This makes a total of sixteen cases of meningitis due to the streptothrix. if we include Naunyn's case which Neuman and Rabinowitsch-Kempner did not include. No attempt has been made to collect the cases of brain abscess either with or without meningitis, but from references to them they are apparently more common than are cases of pure meningitis. Of course as autopsies were not performed in all the cases that we have listed it is possible that a brain abscess may have been present in some of them. However, there was nothing in the clinical histories to indicate that such was the case.

The case that we are reporting gave the following history:—Philip E., 12 years old, had been a blue baby at birth. A heart lesion had been diagnosed during the first year. He had always been extremely delicate. Up to five years of age he had frequent convulsions. His cyanosis was variable in degree but was never absent and he was often prostrated by indispositions that would not have affected a normal child. However, he had adapted himself very well to his physical handicap and had lived a comfortable life. On December 1, 1922, on his return from a visit to Virginia, he complained of headache. On December 2, after going on a shopping trip, he again complained of headache. Neither of these attacks lasted long and they were attributed to the train trip and the shopping. On December 4 he again suffered from headache and on December 5 was seen by his family physician. No cause for the headache could be found. On December 6 he stayed indoors and was in bed part of the time. No definite diagnosis was made. During the next few days he ran a low, irregular fever and vomited once. He was again examined on December 11 and a blood count was done which was reported as follows: Red blood cells, 6,600.000; hemoglobin, 135 per cent.; color index, 99; white blood cells, 10,500; polymorphonuclears, 74 per cent.; small lymphocytes, 24 per cent.; large mononuclears, 2 per cent.; no eosinophiles or basophiles. The urine was also examined and was highly acid but otherwise normal. On the night of December 12 he suddenly became worse. Convulsions developed. On the evening of December 13 he was seen in consultation. He was lying in bed on his left side. His color was quite cyanotic. The face was drawn and there was some flattening on the left side. The tongue was tremulous and could not be protruded. The tonsils were over medium size, very ragged and red. The temperature was 100°F. The right pupil was widely dilated and reacted sluggishly to light. The pulse was fairly regular and full. The rate was 120. The heart was apparently not enlarged. There was a marked systolic and diastolic murmur at the apex; the second pulmonic was accentuated. The respiration was about 60. The examination of the lungs showed a slight general dullness on percussion and scattered moist rales, more numerous at the bases.

There was a marked general hypertonicity of the muscles. The neck was very rigid and so also were the muscles of the abdomen. The knees were drawn up, and the patellar reflexes could not be elicited. The Kernig sign was very marked. A more complete examination was impossible on account of the objection of the mother who was holding the child in her arms.

At about 10 o'clock that evening, when he was seen for the purpose of doing a lumbar puncture, the picture was about the same. At that time there were marked convulsive twitchings of all the muscles. Both pupils were widely dilated and did not react to light. The patellar reflexes were found to be exaggerated. There was a Babinski and an ankle clonus. The pulse at that time was quite irregular, both in rate and in rhythm. A lumbar puncture was done and revealed cloudy fluid under greatly increased pressure. 35 cc. of fluid were withdrawn and 20 cc. of antimeningitis serum injected by gravity. With the aid of a small dose of morphine he passed a fairly comfortable night. On the 14th the condition was about the same. The muscular twitchings were less marked, however. A second puncture was done, no serum being given as the examination of the first fluid showed that the case was not one of meningococcic meningitis. During that night he became much worse and frequent cardiac stimulations were necessary. He died on the afternoon of the 15th.

Examinations of both fluids were practically identical. The fluids were yellowish in tinge and extremely purulent. There was an enormous increase in cells—98 per cent. polymorphonuclears. The albumin and globulin were very greatly increased showing a + + + + massive reaction. The glucose was practically absent as shown by the negative reduction of Fehling's, though the heavy albumin content may have masked the reaction to some extent. The microscopical examination showed numerous microorganisms that appeared to be small gramamphophilic cocci and bacilli. The cultures were negative aerobically on chocolate agar, blood plates, plain agar and broth but anaerobic cultures in a sealed serum broth tube revealed organisms of the same type as those seen in the smear. Not recognizing this organism it was given to Dr. Anna W. Williams who classified it tentatively as belonging to the actinomyces-nocardia-streptothrix group, because of its thread-like forms with swollen ends and its ability to break up into extremely irregular members ranging from minute globoid bodies to larger rounded or oval forms.

After studying the organisms Dr. Williams stated "that the descriptions of most organisms called by any one of these names have been very meager and therefore the classification of the group as a whole is decidedly unsatisfactory. In the first place the name streptothrix for members of this group has been given up by most classifiers because it had already been used for a certain mould. Wright, who wrote the monograph in Osler's Modern Medicine on Actinomyces and similar organisms, revived the term nocardia for those forms that grow freely aerobically, do not form swollen ends in lesions, are Gram-positive and more or less acid fast. He reserved the term actinomyces for strains that produce swollen ends in lesions, grow with difficulty and more or less anaerobically in cultures and are gramamphophilic. In the recent classification recommended by the Society of American Bacteriologists, the term nocardia is dropped and all of the forms including this genus are placed under the genus actinomyces. This genus is placed in the family actinomycetaceae. In this family are included three other genera —actinobacillus, leptotrichia and erysipelothrix. The organism under discussion continued in successive cultures to be strictly anaerobic. On lactose agar it produced abundant branches. It therefore is classified according to the old classification as a streptothrix and according to the latest classification as belonging to the actinomyces."

For bibliography see the original article.

TORULA MENINGITIS

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LOUIS L. SHAPIRO* AND JOSEPHINE B. NEAL

The classification of pathogenic yeasts has been the subject of much discussion among bacteriologists. As a result, the nomenclature has been greatly confused. In recent years it has been shown that one of these forms, *Torula,* has a special predilection for the central nervous system and the lungs. The outstanding study of this organism and its rôle in producing infection has been made by Stoddard and Cutler.

Earlier work was done by Türck and von Hansemann in Germany, and valuable contributions have also been made by Brewer and Wood, Pierson, Rusk, Evans, Freeman and Weidman, Sheppe and Bettin, in this country. These workers have reported thirteen cases that may be considered due to the torula, although the cultural characteristics have not always been entirely identical. Sheppe, in a personal communication, reports another case of meningeal involvement.

This makes, with our own, a total of fifteen cases, of which thirteen involved the central nervous system, one the lung and one the muscles of the back and the vertebral column. Of these thirteen cases of involvement of the central nervous system only five were diagnosed during life by the examination of the spinal fluid. In all cases except our own the diagnosis was made only a comparatively short time before death.

REPORT OF A CASE

History.—A boy aged 16, born in New York, had spent most of his life in New York and New Jersey. He was 6 feet and 1 inch in height and was well developed but slender. He was attending a private school. He had always been well, and there was nothing of moment in his past history until February, 1923, when he had complained of left frontal headache, weakness and sleepiness, which persisted for about one week. At this time he did not appear to be suffering from any acute or chronic disease. His mentality was normal, and physical examination, especially of the nervous system, eyes, ears, nose and sinuses was negative.

Roentgen-ray examination of the head was negative. The temperature, pulse and respiration were normal. Blood pressure was: systolic, 120, diastolic, 68; homoglobin (Dare) 95 per cent.; urine negative. Suspecting encephalitis, he was kept at home another week, at the end of which time he felt so well that he returned to school.

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TABLE 1.

COLLECTED CASES OF TORULA INFECTION IN MAN (AUTHENTIC CASES).

	No. of Cases	Sex	Age	Race	System Chiefly Involved	Result	When Diagnosis Was Made	Duration of Disease
1. Brewer and Wood	1	M	20	Russian	Muscles and vertebral column	Recovered	Organisms recovered	5 months
2. Pierson	1	M	57	American, California	Central nervous system	Fatal	Post-mortem	6 weeks
3. Stoddard and Cutler	1	F	42	American, Florida	Central nervous system	Fatal	Post-mortem	14 weeks
4. Stoddard and Cutler	1	M	39	American, Massachusetts	Central nervous system	Fatal	Post-mortem	12 weeks
5. Rusk	1	M	57	German, California	Central nervous system	Fatal	Post-mortem	10 months? complicated by syphilis
6. Rusk	1	..	..	German, California	Central nervous system	Fatal	Post-mortem	1 month
7. Türck	1	F	43	German	Central nervous system	Fatal	Post-mortem	6 weeks
8. Von Hansemann	1	M	18	German	Central nervous system	Fatal	Post-mortem	?
9. Evans	1	M	13	Mexican, California	Central nervous system	Fatal	Ante-mortem	5 weeks
10. Evans	1	F	20	Mexican, California	Central nervous system	Fatal	Ante-mortem	9 weeks
11. Freeman and Weidman	1	M	39	American, Pennsylvania	Central nervous system	Fatal	Ante-mortem	4 months
12. Sheppe	1	M	48	American, Virginia	Lungs	Fatal	Post-mortem	2 months
13. Sheppe (personal communication)	1	M	..	American	Central nervous system	Fatal	Ante-mortem	Few days ?
14. Bettin	1	F	40	American	Central nervous system	Fatal	Ante-mortem	5 weeks
15. Shapiro and Neal	1	M	16	American, New York	Central nervous system	Fatal	Ante-mortem	7 months

Course of Illness.—He was well until May 15 when, after eating a large quantity of chocolate and pie, he suddenly developed a similar left frontal headache with vomiting, weakness and dizziness. These symptoms persisting for several days, he returned to New York, and was again seen on May 19. Not responding to symptomatic treatment, he was sent to a sanatorium on May 23. Physical examination at this time was negative, except for a loss of 15 pounds (6.8 kg.) in weight since February. Mentally, he was normal until the evening of May 25, when he suddenly became delirious. This lasted but a few hours. Neurologic examination at this time showed exaggeration of both patellar reflexes only. On May 27 the patient complained for the first time of pain in the back of the head and neck, slight disturbance of vision and photophobia. From then until May 29 evidence rapidly developed that we were dealing with an acute infection of the central nervous system, particularly of the meninges. He showed definite rigidity of the neck. The eyes were painful on extreme movement to the left, the pupils were equal in size and reacted normally to light and accommodation. The fundi showed beginning choked disc slightly more developed on the left. There was no definite evidence of cranial nerve involvement. The abdominal and cremasteric reflexes were diminished, especially the right. Both knee reflexes were markedly diminished. The bilateral Kernig sign was positive. Mentally, he was clear but somewhat irritable. The temperature was 101 F., pulse, 52; respiration, 20 and regular. The temperature and pulse previous to this time had always been normal. A cell count revealed: red blood cells, 5,640,000; hemoglobin, 89 per cent. (Dare); white blood cells, 14,400; polymorphonuclears, 80 per cent., and lymphocytes, 19 per cent.

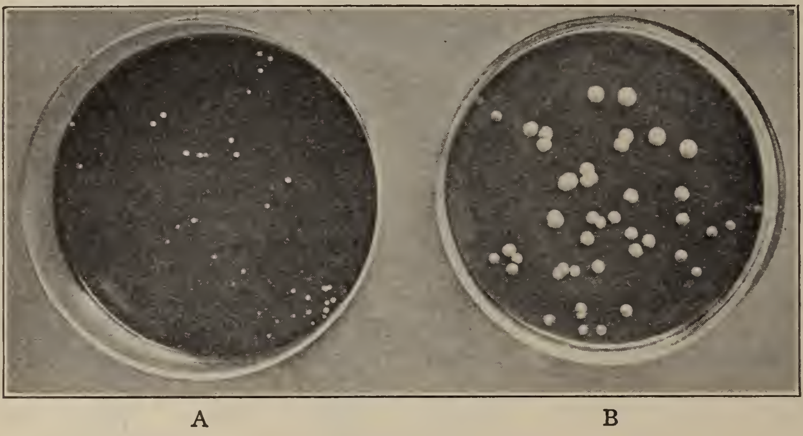

Fig. 1.—*A,* forty-eight hour culture of Torula on glucose veal agar plate; *B,* seven day culture. (Reprinted by courtesy of Archives of Neurology and Psychiatry.)

Lumbar puncture was performed for the first time on May 29. The fluid was found to be under very high tension, and slightly hazy in appearance. There was no growth in the culture of the fluid the first eighteen hours, but distinct yeast colonies were apparent on the following morning, May 31, thereby establishing the diagnosis of a yeast infection of the central nervous system. Lumbar punctures were subsequently made daily until near the end, and the torula organism was recovered from the fluid by culture and observed in smears.

From May 29 until June 18 there was evidence of rapid progress of the disease, with symptoms of increasing intracranial pressure. The patient was frequently delirious and lost weight rapidly. He complained of photophobia and double vision. Rigidity of the neck persisted. The pupils were moderately dilated and were sluggish to light. Distinct weakness of the right and left external recti developed. The fundi showed increasing papilledema, being from 2 to 3 diopters on June 2, and 4 or 5 diopters on June 7. The abdominal and cremasteric reflexes were not elicited. The deep reflexes were generally diminished. A bilateral Kernig sign was present, but no Babinski sign. The temperature was irregular, varying from 98.6 to 103.8 F. The pulse was always slow in comparison with the temperature, ranging from 60 to 100 and averaging about 75. Respiration was normal. On the morning of June 8, the patient went into a deep stupor. Toward evening, he gradually roused after another lumbar puncture and tube feeding, and was rational again the next morning. Tube feeding was necessary from this time on. This was done every six hours, and continued until about the middle of August. In this way, the caloric intake for twenty-four hours was increased from less than 800 to more than 5,000. Up to this stage one lumbar puncture daily was sufficient to relieve intracranial pressure. Now, however, a puncture would afford relief for only about ten hours, when the patient would again become restless, irrational, noisy and cry out as if in great pain. Punctures therefore were made twice a day until June 19, when one puncture again gave sufficient relief.

Tube feeding was discontinued about the middle of August, from which time he took nourishment well, averaging about 5,000 calories in twenty-four hours. He gained considerably in weight and appeared to be well nourished. He was quiet and seemed comfortable most of the time. Mentally, he was for the most part lethargic, confused and disoriented. Frequently, there were periods lasting several days or weeks when at times he seemed quite rational, would talk coherently when spoken to, would recognize people, and appeared to be interested in what was going on about him. He rarely complained of headache and vomited only three times. Weakness of the right and left external recti persisted throughout. The fundi improved considerably, showing an elevation of 1 diopter in the right disk and 1.5 diopters in the left. The margins appeared fairly distinct, and the veins were less tortuous and full. No further cranial nerve involvement appeared. The abdominal and other superficial and deep reflexes remained absent throughout. Kernig's sign was always present. No gross sensory or motor changes were obtained. The temperature was always irregular, showing a morning fall and evening rise. From the middle of June to early in July it varied from 101 to 103.6 F., occasionally reaching 104 and once 105 degrees. During the remainder of July it varied from 100 to 102 F., except for a brief interval. During August and September it varied most of the time between 99 and 101 F., occasionally reaching 102 degrees during September.

The pulse gradually increased in rate, varying during the last half of June between 100 and 120. During July, it ranged between 110 and 130. During August and September, it varied between 130 and 150, averaging about 140. The respirations were always normal and regular. During this entire period the lungs were clear and the heart was normal except for the tachycardia. The blood pressure varied from time to time between 90 and 144 systolic.

During the last week in September scattered, moist râles were detected at both bases posteriorly. On October 1 the patient again became stuporous.

The lungs showed impaired resonance with small areas of distant, high pitched breathing. Pulmonary edema developed on October 8 and the patient died on the evening of October 11 of respiratory failure.

Peculiar pressure sores developed over both hip joints, the inner side of both knee joints and both heels during the last month of the disease. They began as large violet-blue blebs, rapidly breaking down, becoming excavated, but with firm indurated margins. Cultures taken from these areas were negative for torula organisms.

Treatment.—As the smear from the first spinal fluid withdrawn showed forms suggesting gram-negative cocci, antimeningococcic serum in 20 cc. doses was given intraspinally the following four days. When the diagnosis of yeast infection was definitely established, it was decided to give iodids because of their action on other yeast infections, especially the oidiomyces. Sodium iodid, 15½ grains (1gm.) was therefore given intraspinally every other day, beginning June 3, until July 19. One hundred grains (6.5 gm.) of sodium iodid was also given daily internally, beginning May 31, and increased to 400 grains (26 gm.) daily from August 1 to the end of September. The iodids were given again intraspinally every other day during August. The patient tolerated the iodids very well, and at no time showed

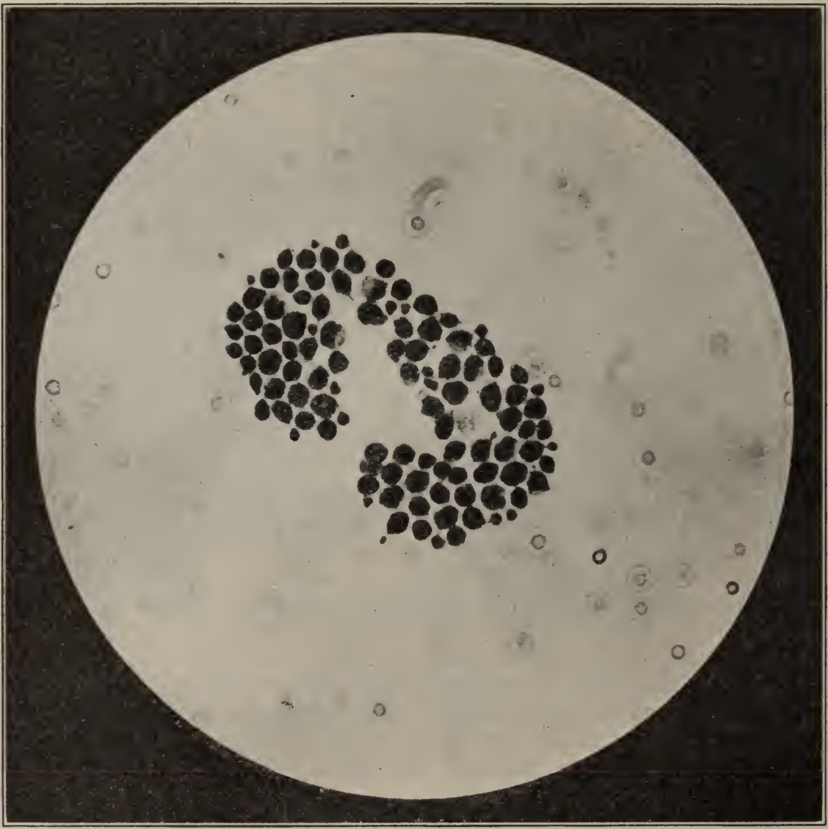

Fig. 2.—Smear of fifteen day culture of *Torula;* budding forms.
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symptoms of iodism. Because of the favorable reports from the use of monilia vaccine in tropical sprue, a vaccine was made from the torula organisms and given twice weekly, beginning June 6 with 500,000, increasing 500,000 with each dose. This was continued throughout the entire course of the disease. Beginning August 25, vaccine prepared by the Dreyer method was used. The patient never showed any local or general reaction.

On July 21, 24 and 26, a colloidal silver preparation was given intraspinally. Each administration was followed in a few hours by a thermic reaction, the temperature rising to 103 F., and gradually declining to 99 F. in forty-eight hours. This was tried on account of a great increase in the number of colonies in cultures. After the first injection there was also a severe general reaction. As there was no evidence of improvement in the patient's condition or of effect on the growth of the organisms, the colloidal silver preparation was discontinued after the third dose.

Serum obtained from rabbits immunized by intravenous injection of the torula was given intraspinally on September 5, 8 and 13, the first dose being 5 cc., the second 3 cc., and the third 5 cc. The third dose was followed by a severe general reaction, with a temperature of 104 F., pulse 170 and respiration 30, so that it seemed inadvisable to continue giving the serum.

Arsenic was given internally in the form of liquor potassii arsenitis (Fowler's solution), 15 minims (1 cc.) daily during the month of August.

A high caloric diet averaging about 5,000 calories in twenty-four hours, well proportioned in proteins, carbohydrates, fats, fruit juices and salts, was given and well tolerated throughout the entire course of the disease. As mentioned previously, tube feeding was resorted to for a period of about two months.

Clinical Pathology.—The Wassermann test was negative on the blood and the spinal fluid. Blood cultures taken June 7 and 29, August 21, September 23 and October 5 were all negative for *Torula*. Complete blood counts were made every ten days throughout the course of the disease. The average count for June was: hemoglobin, 80 per cent. (Dare); red blood cells, 4,840,000; differential count normal; color index, 0.83; white blood cells, 12,400; polymorphonuclear neutrophils, 83 per cent.; lymphocytes, 13 per cent.; large mononuclears, 4 per cent.; eosinophils, none.

On June 8, when the patient was in deep stupor, the white blood cells were 28,000 with 94 per cent. polymorphonuclears. The average counts for July, August and September were about the same. On October 10, the examination showed for the first time qualitative changes indicative of anemia.

The blood chemistry on June 11 was within normal ranges. The findings were; nonprotein nitrogen, 34 mg. per 100 cc. of blood; urea nitrogen, 15 mg. per 100 cc. of blood; uric acid, 2.5 mg. per 100 cc. of blood; blood creatin, 1.5 mg. per 100 cc. of blood; blood sugar, 100 mg. per 100 cc. of blood; whole blood chlorid 363 mg. per 100 cc. of blood; alkali reserve, 70 per cent.

On August 16 an agglutination was made with the torula and the patient's serum, undiluted, and in dilutions of 1:10, 1:50, 1:100 and 1:500. There was no agglutination, although the patient had been receiving vaccine for more than two months. Agglutinations in the same dilutions were made with horse and sheep serum. It was hoped that one of these animals would show some natural immunity to the torula, but none was found.

Throat cultures and cultures of the sputum were negative for torula organisms.

Urine analyses were made practically daily throughout the disease. The average analysis was: specific gravity, 1,023; albumin, trace, with few medium to large finely and coarsely granular and hyaline casts; sugar, acetone and diacetic acid, none.

Cultures of the urine from time to time were negative for *Torula* organisms. The patient had a urethral discharge during September. Cultures from this were negative for *Torula* organisms and gonococci.

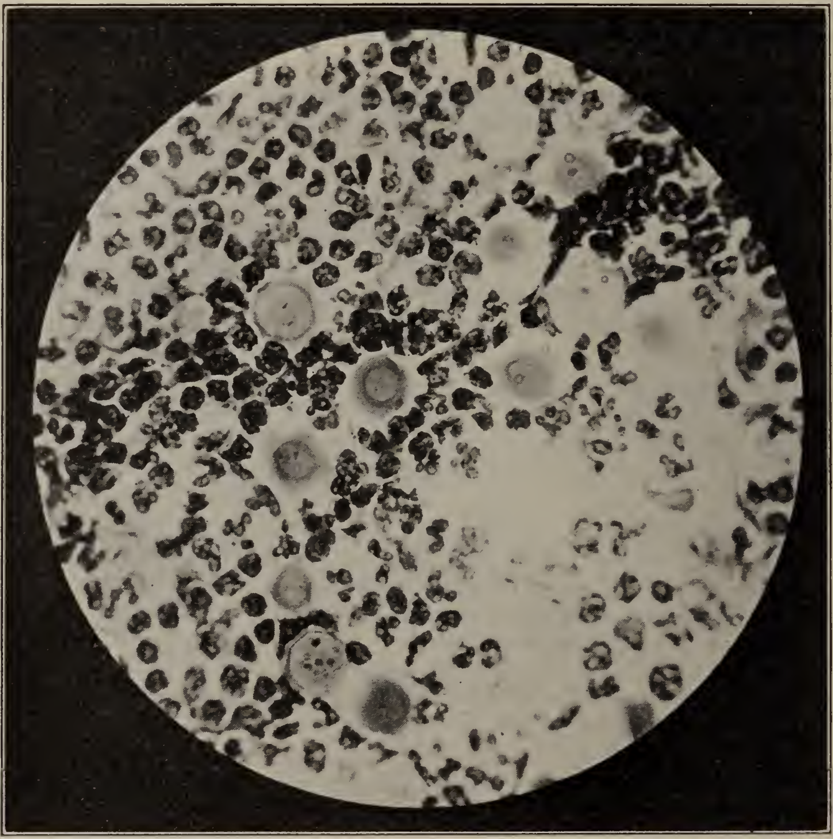

Fig. 3.—Smear of ninth spinal fluid, showing forms of various sizes. Rings of cytoplasm vary in width. Nuclear material is granular in some forms.
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Spinal Fluid Examinations.—The first lumbar puncture was performed on May 29, 1923. The fluid was under greatly increased pressure and was slightly hazy and showed 300 cells per cubic millimeter. Of course, many of these were yeast cells. The other cells were about 90 per cent. mononuclears. Smears were stained both by the Ziehl-Neelson stain and by the Gram method. No tubercle bacilli were found in the Ziehl-Neelson stain, but there were many large heavily stained cells which were later identified as yeast cells. In the smear stained by the Gram method there were seen degenerated cells, with a suggestion of intracellular gram-negative cocci. There was a moderate increase (+ +) in albumin and globulin in the fluid, and the sugar-content as shown by the reduction of Fehling's solution was markedly diminished (+).

The second lumbar puncture, made May 30, revealed 45 cc. of hazy fluid, with practically the same findings as in the first, except that the percentage of polymorphonuclear cells was considerably higher.

On May 31 there was a heavy growth of yeast on the forty-hour culture from the first fluid and a light growth on the twenty-four hour culture from the second fluid. The smears and cultures were submitted to Dr. Anna W. Williams, Assistant Director of the research laboratory.

Daily lumbar punctures were performed until June 8. At each puncture from 30 to 60 cc. of fluid were withdrawn. The chemistry of the fluid remained about the same, but the type of cells was apparently modified somewhat by the administration of serum, as fluids withdrawn after serum was given usually showed a high percentage (80 to 90 per cent.) of polymorphonuclears. A Wassermann test on the second fluid was negative. The *p*H of a number of fluids was ascertained. It ranged from 7.5 to 7.8, which is within the normal limits for spinal fluid and indicates that the torula was not rendering the fluid acid. In the first four fluids the yeast cells were large and stained heavily. The structure was not well shown. They stained best by the Ziehl-Neelson method. With the Gram stain they were amphophilic and less easily distinguishable from the cells of the spinal fluid. After the fourth puncture, the structure of the torula was better shown. The organisms varied greatly in size and appearance. In some, the margin was sharply defined; in others, the outline was indistinct and fuzzy. In some, the greater part of the cell was made up of a densely staining nuclear-like material surrounded by cytoplasm of varying widths. In others, this densely staining nuclear substance was relatively small and surrounded by a wide clear cytoplasm. At times, the center of the cell was apparently broken up into granules, which often took a reddish stain. Budding forms were seen on practically all types of cells. Small forms with practically no cytoplasm might have been mistaken for small lymphocytes if buds had not been seen. The cytoplasm was apparently of a gelatinous nature, as shown by the fuzzy halo. This gelatinous character may also account for the fact that the yeast cells, especially those with a small nucleus and a wide cytoplasm, were often almost completely surrounded by lymphocytes. The gelatinous character of the cytoplasm or capsule has also been noted by Sheppe, who observed that in contaminated cultures the yeast cells were often surrounded by other organisms. In all of these early cultures there was a luxuriant growth in from twenty-four to thirty-six hours.

From June 8 to June 18 inclusive, two punctures were made daily for the relief of pressure. From 40 to 60 cc. of fluid was usually withdrawn at each puncture. These fluids yielded practically the same findings as before. On June 15 the fluids became somewhat clearer, with a corresponding decrease in tissue cells—although they remained greatly increased above the normal. The relative proportion of mononuclears and polymorphonuclears often changed for no apparent reason. The number of yeast cells varied from time to time. On June 16 the culture began to be less luxuriant, and from that time the number of colonies gradually decreased in number and did not appear until forty-eight hours had elapsed. Beginning with June 19, daily punctures were made again. The character of the fluid showed little change, but the colonies continued to diminish in number, and on June 21 did not appear until after seventy-two hours had elapsed. The number of colonies was small, even on plates inoculated with centrifuged sediment. For some time plates had been made with both the centrifuged and uncentrifuged fluid to estimate as well as possible the number of yeast cells. It was noted that there was little relation between the number of colonies and the number of yeast cells seen in the smear. Often when only two or three yeast cells could be found in the smear there was an abundant growth, and we therefore concluded that it was impossible to differentiate the small form of the torula from the lymphocyte. This decrease in the number of colonies and their rate of growth continued until July 16, when the cultures again began to show in forty-eight hours and the number of colonies began to increase rapidly. On August 1 the colonies grew in twenty-four hours. From this time the colonies varied in rate of growth and in number, but they were, on the whole, far less numerous than during the last two weeks of July. The cultures continued positive until September 26, although the number of colonies was small. The cultures on that date were negative, as were also the succeeding five cultures. The character of the fluid remained much the same until August 22, when the fluid became slightly blood-tinged. On August 26 the fluid was yellowish, with no free blood present. This condition continued for some days, when the fluid again became slightly blood-tinged, and thereafter

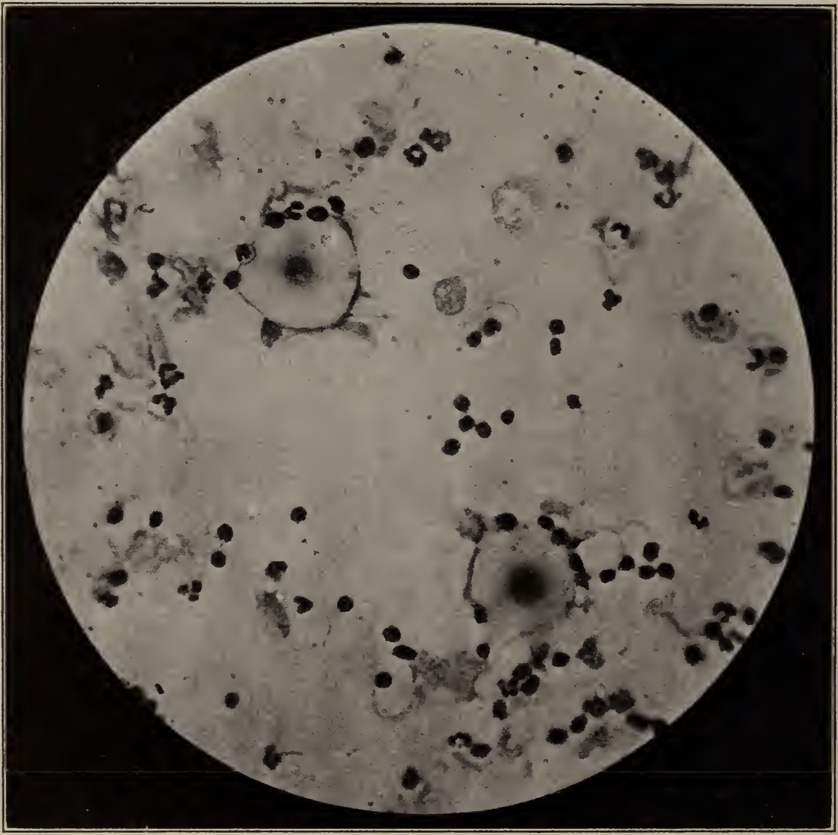

Fig. 4—Smear of twenty-fourth spinal fluid showing *Torula* surrounded by lymphocytes. (Reprinted by courtesy of Archives of Neurology and Psychiatry.)

was either blood-tinged or yellowish until the end. The amount of blood present apparently was not sufficient to affect greatly the protein content, since the quantity of albumin and globulin gradually decreased from a moderate (+ +) to a slight (+) amount by the end of August. The amount of sugar gradually increased and was apparently normal by the middle of August.

On September 6, after an intraspinal injection of rabbit serum, the quantity of albumin and globulin increased, and the percentage of cells changed from 70 to 80 per cent. mononuclears and to 70 per cent. polymorphonuclears. On September 14, following the reaction to the rabbit serum, the quantity of sugar became suddenly greatly diminished, but subsequently increased to normal and remained so until the last fluid withdrawn on October 8, when it again fell off slightly.

From time to time a web appeared in the fluid. At times it was fine, resembling the web in the fluid from a case of tuberculous meningitis. But at times it was dense and gelatinous, with many branches extending from a central filament to the sides of the tubes.

In all, 133 lumbar punctures were performed. Except at the last two punctures, when a higher level was used, the punctures were made in three interspaces—between the fourth and fifth, third and fourth and second and third lumbar vertebrae, and the back remained in excellent condition. Except for some staining of the skin from the iodin, there was little indication that it had been submitted to such a long-continued ordeal.

The cultures grew readily on ordinary agar either with or without the addition of blood. The *p*H of the mediums did not seem to affect the growth. Mediums of various pH values were tried, ranging from 5.8 to 8.6, with no apparent effect on the growth. We used 2 per cent. glucose veal agar, *p*H 7.6. The cultures grew equally well at room and at incubator temperatures. When the colonies first appeared they were pin-point in size and increased rapidly to perhaps a centimeter in diameter. The discrete colonies were uniformly rounded, with a sharply defined margin and a high center. They appeared moist and glistening, and did not adhere tightly to the medium. In consistency they were soft and not stringy. On slants, they coalesced, forming a thick moist, creamy growth. In color, they were a very pale creamy yellow at first, becoming darker yellow as they grew older. The tops of the older colonies were darker than the rest. They grew less well in beef extract broth, *p*H 7.5, than on solid mediums. They clouded the broth slightly and formed a heavy, creamy sediment in the bottom of the tube. There was no pellicle formation. The cultures did not grow anaerobically. No mycelial formation was noted. The action on sugars was studied by surface and stab cultures in veal agar *p*H 7.4 containing 1 per cent. of the various sugars and 1 cc. Andrade. There was no formation of either gas or acid at the end of more than thirty days in the following sugars: lactose, levulose, mannite, glucose, saccharose, inulin, dextrose, starch and glycerin.

The smears of the cultures were gram-positive in the earlier generations, but smears made from cultures several months old showed many gram-negative forms. In the earlier cultures, the organisms had a deeply staining center, with a narrow ring of less deeply staining cytoplasm. While they varied greatly in size, no large forms, such as were found in the smears of the spinal fluids, were ever seen. In the older cultures, the central material was broken up into granules. Many budding forms were seen in the cultures. There was no evidence of endospores.

The absence of endospores, the reproduction by budding, the absence of mycelium and the failure to form gas in sugars identify the yeast as a torula.

Park and Williams give the following tentative classification of the yeast and so-called intermediate groups.

Somewhat rough studies were made in an effort to find some substance that might have an inhibiting effect on the growth of the torula, with the following results: sodium iodid, 1:500, no inhibition; sodium salicylate, 1:500, no inhibition; quinin, saturated solution, no inhibition; a colloidal silver preparation 1:600, 1:1,000, 1:6000, slight inhibition; magnesium sulphate, 1:500, no inhibition; rochelle salts (tartrates) 1:500, no inhibition; tricresol, 1:1,000, some inhibition; acriflavin, 1:1,000, 1:5,000, complete inhibition, 1:10,000, well marked inhibition. Acriflavin was the only substance of the series which had any definite effect on the growth of the torula, but it was not used in treatment.

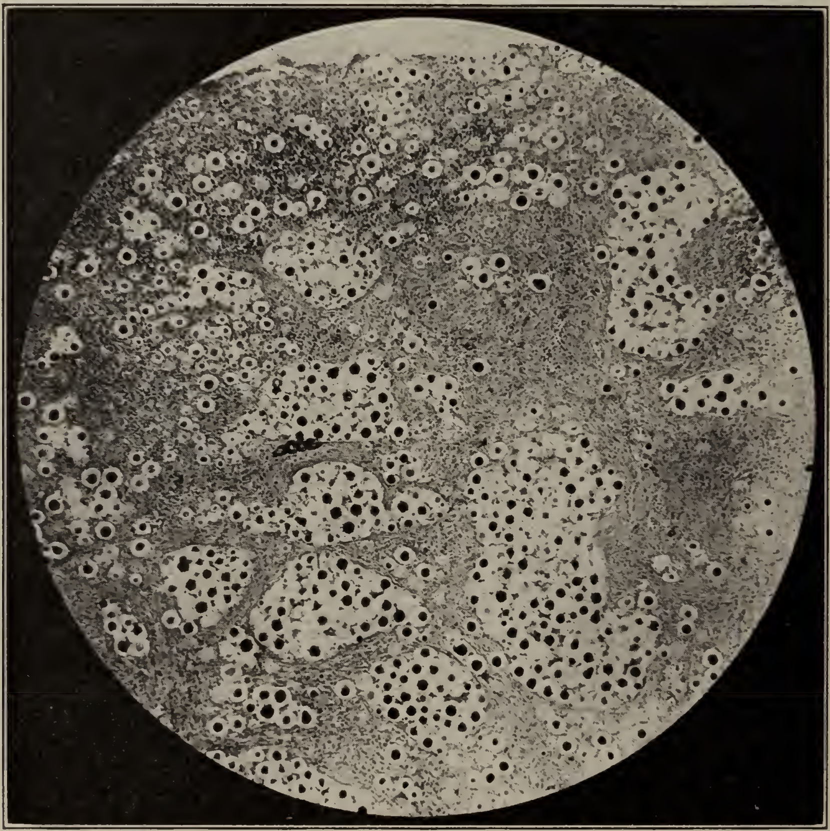

Fig. 5.—Low power magnification of section of subcutaneous mass, guinea pig 644, showing *Torula* arranged in groups. Eosin-methylene-blue stain.

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ANIMAL INOCULATION

On June 8, 1923, eight young animals, four rabbits (about 1,500 gm.) sion of a forty-eight hour culture of the torula as follows: Rabbit 86, one-quarter cc. intracranially; Rabbit 36 one-quarter cc. intracranially; Rabbit 621, five cc. intraperitoneally; Rabbit 829, three cc. intraperitoneally; Guinea-pig 657, one-quarter cc. intracranially; Guinea-pig 655, one-quarter cc. intracranially; Guinea-pig 647, three cc. intraperitoneally; Guinea-pig 653, two cc. intraperitoneally.

Rabbit 86 died July 17, 1923, and no torula were found by culture in any of the organs.

Guinea-pig 657 died June 17, and necropsy was not performed. Guinea-pig 653 died June 22, and showed no torula in the cultures.

Rabbit 829 seemed sick about August 27. This animal had been inoculated intraperitoneally about eleven weeks previously. There was a skin lesion on the back over the lower part of the spine, with superficial ulceration and crust formation. He had general muscular tremors, was ataxic in his movements and seemed to be blind. He was chloroformed and necropsy performed on August 30 by Mary Nevin. Just before death a lumbar puncture was performed, and about 1.5 cc. of spinal fluid withdrawn, which was hazy, showed a slight increase in mononuclear cells and in albumin and globulin and a normal sugar content. The smear showed a few torula and other organisms. The torula was finally recovered in pure culture from the fluid and also from the skin lesion, brain, liver, spleen, lungs and kidney. The organs in the gross showed no definite lesion. The sections showed no large forms of torula, but a study of the sections showed small forms similar to those in the culture scattered through the organs, especially in the periphery of the organs and in the lymph spaces of the brain.

TABLE 2.

PARK AND WILLIAMS' CLASSIFICATON OF YEASTS

	Genus	Endospores	Budding	Mycelium	Gas Formation
Blastomycetes..	Saccharomycetes ...	+	+	−	±
Fungi Imperfecti ...	Torula	−	+	−	−
	Monilia	−	+	±	+
	Oidia	−	+	+	−
	Coccidioides	+	−	+	−

Rabbit 36 died on October 16. It had been inoculated intracranially more than four months previously. It had not appeared sick at any time. It had apparently grown normally since the inoculation and was well nourished. On necropsy, the brain apparently was normal. The liver was pale, mottled and brittle. The spleen was enlarged and very dark. The kidneys were normal. The lungs showed edema, with some hemorrhagic spots and congestion. The torula was obtained in pure culture from the brain, liver, spleen, lungs and heart. The plates were inoculated by smearing sections of the organs over them. The colonies were numerous, and there was no contamination. As in Rabbit 829, no large forms of torula were found in the sections of the brain, but small forms were found as in Rabbit 829. The other organs were not sectioned. It will be observed that in Rabbit 829, inoculated intraperitoneally, the torula had invaded not only the abdominal viscera and lungs, but also the spinal fluid and brain. In Rabbit 36, inoculated intracranially, the torula had invaded not only the brain, but also the abdominal and thoracic viscera. It is also interesting to note that Rabbit 36 lived more than four months and, in spite of the widespread distribution of the torula, grew normally in size, was exceptionally well nourished and showed no evidence of infection up to the time of his death. The fourth rabbit apparently escaped or overcame the infection. He died, Jan. 18, 1924, over seven months after inoculation, and no torula were recovered by culture from any of the organs.

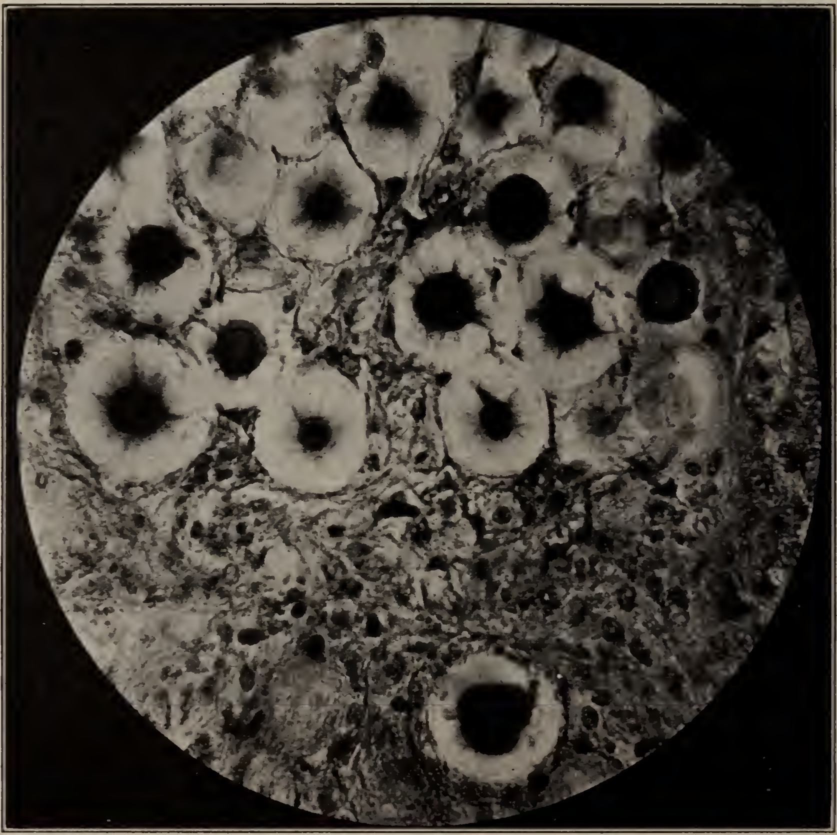

Fig. 6.—High power magnification of section in Figure 5. There are numerous *Torula*—a few budding forms. (Reprinted by courtesy of Archives of Neurology and Psychiatry.)

The two guinea-pigs that survived grew rapidly and seemed perfectly well. They were kept under observation, however, and on November 3, more than five and one-half months after inoculation, it was noticed that Guinea-pig 655, inoculated intracranially, showed spasmodic contraction of groups of muscles, and held the head turned markedly to one side. He was weak in in his movements and was unable to stand normally. He was panting for breath. He was chloroformed on that day and necropsy was performed. The brain was edematous and soft, and the fluid oozed out of it. The right lung was small and shrunken; the left was swollen. The spleen was small and mottled. The stomach was empty and distended with gas. The other organs seemed normal. The torula was obtained in pure culture from the brain, lungs, liver, spleen, kidney and heart. The plates were inoculated by smearing with pieces of the organs. The colonies were numerous, and the culture showed practically no contamination.

In the sections from Guinea-pig 655, many large forms of the yeast were found, especially in the lymph spaces of the brain and in the meninges, and also in the lung (Figs. 7 and 8). It will be noted that budding forms are also seen.

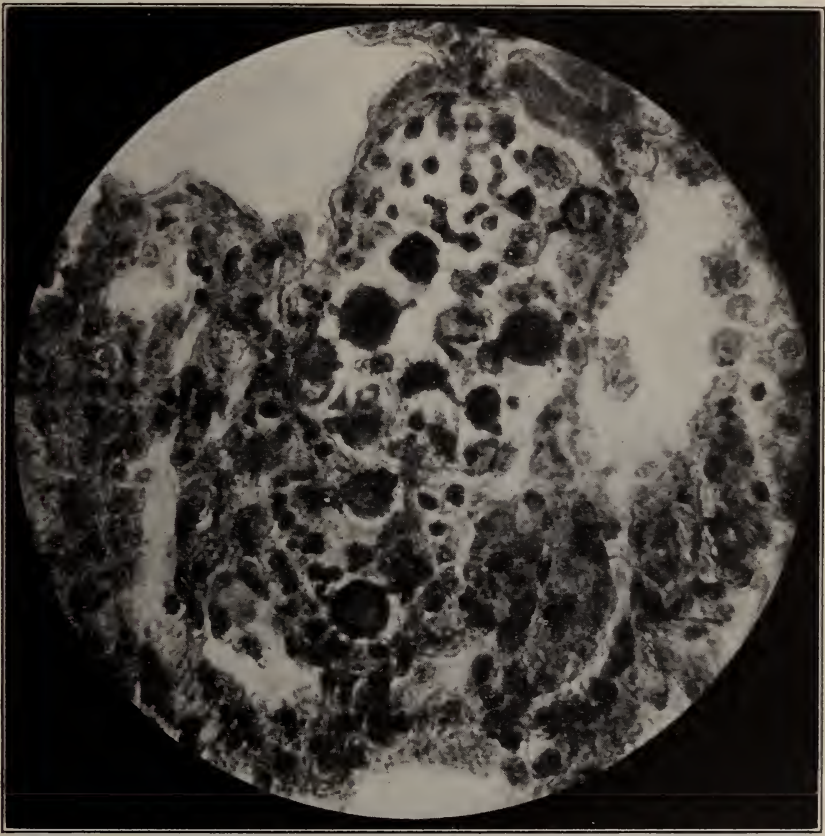

Fig. 7.—High power magnification of section of lung of Guinea-Pig 655. There are numerous *Torula;* a few budding forms.
(Reprinted by courtesy of Archives of Neurology and Psychiatry.)

Guinea-pig 644 seemed normal until late in December, when a hard mass was observed in the left abdominal wall. Soon after that, small ulcerative lesions were observed on the right lower eyelid and on the right cheek. He was chloroformed and necropsy performed on Jan. 3, 1924, nearly seven months after the intraperitoneal inoculation. The brain and other organs seemed normal, and no torula could be cultivated from them. The torula was cultivated from the mass in the abdominal wall, and from the two skin lesions. The plates were inoculated as in the previous cases. The colonies were numerous and the culture practically pure. All these cultures were tested with the sugars (mannite, glucose, glycerin, starch, saccharose, dextrin, inulin, lactose and levulose) and gave no fermentation with any of them. The mass in the abdominal wall was located in the subcutaneous tissue between the skin and the abdominal muscles. It was about 2 inches in length and 1 inch in diameter. It was irregularly spindle-shaped and about the consistency of paraffin. In the sections of this mass many large forms of torula were present. The arrangement in masses is shown in Figures 5 and 6. It will be noted that there is practically no cellular reaction accompanying the invasion with the torula. The torula was much better shown by the eosin-methylene blue stain than by the hematoxylin-eosin.

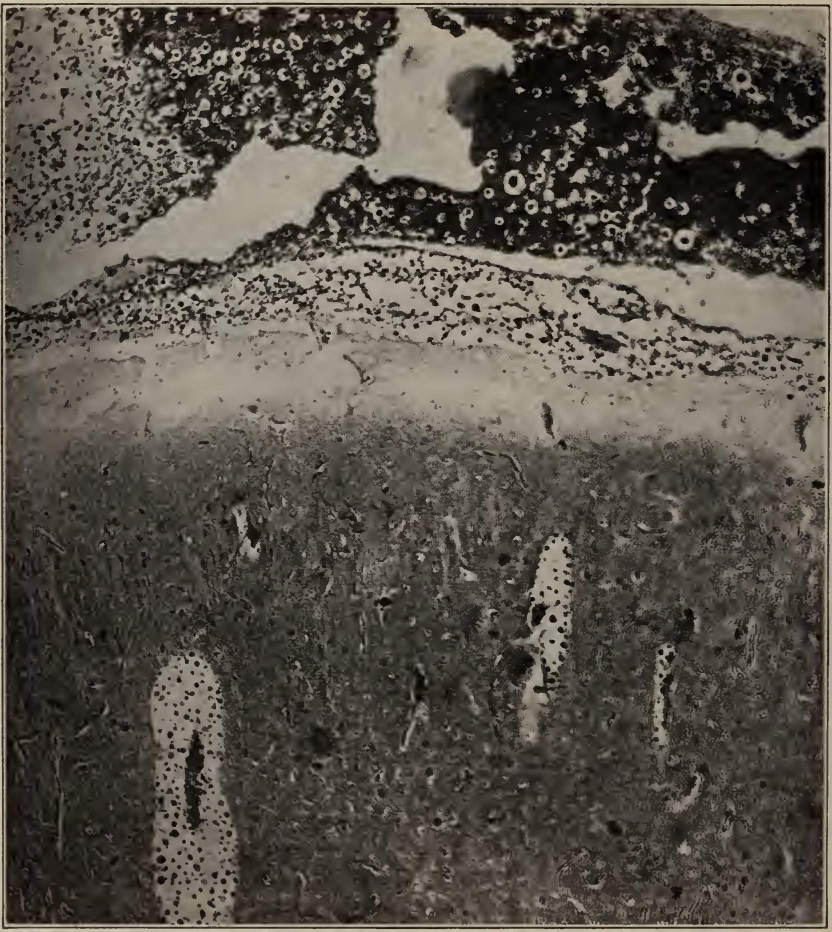

Fig. 8.—Low power magnification of section of brain of Guinea-Pig 655, showing *Torula* in lymph spaces around blood vessels and in meninges. Eosin-methylene blue stain. (Reprinted by courtesy of Archives of Neurology and Psychiatry.)

CONCLUSIONS

In comparing our case with those reported by others, it may be pointed out that we were fortunate in having the patient under our observation during the entire course of the illness. The diagnosis was made within the first two weeks of the acute illness and within five days of the onset of symptoms involving the central nervous system. It is possible that the real onset of the infection was in February, with a period of quiescence lasting until May. He lost 15 pounds in weight during this time, although otherwise apparently well.

Various methods of treatment were tried, apparently with no effect. Arsphenamin had been used by Stoddard and Cutler without success in one of their cases, and was not tried in our case. Decompression was considered, but the repeated lumbar punctures seemed to afford sufficient relief of pressure.

In spite of the fact that the boy was acutely ill for five months, with a constant fever, it was possible to keep him exceptionally well nourished until the very end, and there were very few focal symptoms, although the disease must have been constantly progressing. These same points were observed in the animals inoculated as has already been mentioned.

From a study of the patient and the animals inoculated, infection with this torula seems to be unusually slow and insidious in its course, and inevitable in its outcome.

For bibliography see the original article.

A CASE OF SPOROTRICHOSIS MENINGITIS

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George H. Hyslop, Josephine B. Neal, Walter M. Kraus and Oliver Hillman

Introduction

We wish to report this case of chronic meningitis because the spores constantly found in the spinal fluid of the patient were morphologically similar to the sporotrichia of Schenck and de Beurmann. We feel satisfied that these organisms were the specific cause of the infection, and that our diagnosis was warranted, although we failed to obtain positive cultures and our animal inoculation experiments were inconclusive.

Sporotrichosis in man was first described by Schenck in 1898. Following him, de Beurmann and Gougerot reported several cases in 1900. Since then various reports have appeared, so that the disease has become well established and easily recognized clinically.

The infection is due to the sporotrichia, an organism allied to the hyphomyces. It is pathogenic for horses, dogs, rats, rabbits and guinea pigs. In man, it commonly produces skin lesions which are gummatous in character and may be mistaken for syphilitic or tuberculous lesions. The swellings are usually painless, slow in growth, chronic in nature, and they may either show a central necrosis, eventually breaking down with sloughing of the overlying skin, or they may resemble indolent ulcers.

The infection may become systemic, and it has been reported to have involved the lungs, bones, periarticular tissues, kidneys, and the eyes, causing miliary conjunctivitis and occasionally panophthalmitis.

Involvement of the central nervous system in man has never been reported. Dominguez, however, cites a case of an extradural abscess, complicating a sporotrichosis infection of the middle ear.

The organism is difficult to find in the tissues, but when present is pleomorphic. In smears from the tissues, the sporotrichia may be seen as round or oval bodies with dimensions from 1 to 2 micra by 3 to 5 micra, and appear occasionally in bacillus-like forms.

It grows at room or incubator temperature, and best in sugar media (dextrose, levulose and maltose). Sabouraud's medium is commonly used. It is also reported to grow in 1 per cent. acid broth. First cultures may be slow in appearance and may not be visible for as long as two weeks. They are white or dirty gray on solid media, but if the blood media are used, the cultures may be brown or black pigmented. Microscopically, the growth usually shows branching filaments, with spores in the filaments or in the interspaces. Occasionally the filaments radiate as they do with actinomyces. Subcultures grow more luxuriantly.

A definite diagnosis is made by positive cultures. Complement fixation, agglutination, and skin reaction tests have diagnostic value.

Iodides are specific for the disease.

CASE REPORT

Past History. The patient was a girl aged fifteen years, of American stock and a resident of Bayonne, New Jersey. She had had mumps, measles and scarlet fever, and in 1923 she suffered from what was called a mild attack of malaria. Menstruation began at the age of thirteen years, and was normal in character.

During the fall of 1924 she was in school and in perfect health. Just before Christmas she fell down a flight of stairs, without injury other than bumping the back of her head; she was not even stunned.

Present Illness. On December 30, 1924, she became ill with nausea, vomiting, headache, generalized abdominal pain, and a fever which was not higher than 103°F. In a few days, photophobia, weakness of the right side of the face and intense drowsiness developed. The fever disappeared after three or four days, but the nausea and vomiting coninued, and her headaches were quite severe.

About January 15, 1925, diplopia, strabismus, and lancinating pains in the shoulders appeared. During the next month, symptoms fluctuated from day to day, with a trend toward improvement. The girl could not walk well, because of a tendency to stagger to the left, and any sudden change of position was apt to cause projectile vomiting. She was emotionally unstable, and had frequent nightmares.

She was examined in consultation on February 19. She seemed perfectly normal mentally, and coöperated well. The pulse was 100, there was no fever, and body nutrition was unimpaired. There were no signs of visceral disease.

She preferred to lie in bed, but could sit up comfortably if she moved carefully. With one eye covered she could walk perfectly well.

Cranial Nerves. There was obliteration of both optic disks and moderate engorgement of the retinal vessels; both pupils were large and irregular, but reacted to light and accommodation normally. The left external rectus muscle was completely paralyzed, and the right was partially so. There was a mild lower neurone type weakness of the right facial muscles. Otherwise the cranial nerves were normal.

Motor System. All tests were performed well, except for a cerebellar incoördination in the left arm and leg.

Sensory System. No abnormalities were found.

Reflexes. Brudzinski and Kernig signs were positive. The arm and abdominal reflexes were normal. The knee and ankle jerks were abolished, and the plantar responses were sluggish and equivocal in type.

From February 19 to March 5 there was a steady improvement. There was almost no headache, no drowsiness, a decrease in vomiting, and she was able to go about the house. On March 5, a day or two after some difficulty in naming persons and objects had been observed, she vomited several times. While talking in the afternoon with some friends, she suddenly cried out, saying "I can't, I can't," and in a moment became flushed, clenched her arms and was unable to speak. In a few minutes she recovered sufficiently to get to her room. There was considerable vomiting for several hours, and it was 10:30 p.m. before she could express herself in speech. She seemed frightened, misnamed her mother, and talked incoherently of being cut and hurt. By the next morning she was normal mentally and showed no aphasia, but there was considerable headache, and projectile vomiting occurred if she moved about in bed.

Examination on March 6 showed that there had been an improvement in the external rectus palsies with no change in the other abnormalities found at the time of the first examination. However, there were three new signs—a left partial ptosis, tinnitus in the left ear, and exudate in the left retina. The retinal vessels were moderately dilated, the disk was not visible, and radiating from the edges of the disk were large splashes of pure white exudate, extending outward into the retina a distance of perhaps two disk diameters. There was enlargement of the blind spot in the left eye, but no impairment of the visual fields. The right fundus was normal. There were no evidences of an aphasia or other residuals of the attack.

On March 10 another attack with confusion and a loss of speech, occurred. The patient had been drowsy the day or two preceding. She was then admitted to the Neurological Institute.

Lumbar puncture was done, and about 40 cc. of fluid was drawn. It was clear and under intense pressure. Physical examination revealed increase in the right facial weakness, and much impairment of vision in the left eye, so that fingers could be counted only when held at a 6-inch distance. The neck was quite stiff. Otherwise there were no changes since the examination of March 6.

Blood count showed 21,000 white cells, of which 82 per cent. were polymorphonuclear leukocytes. The urine was normal.

The spinal fluid contained two cells and 160 spores per cubic millimeter. Globulin and albumin were 2 +; and sugar, 68 mg. per 100 cc. Serological tests were negative. A smear of the centrifuged fluid, stained with methylene blue, showed abundant round and oval bodies which took the stain well and in many instances contained what seemed to be polar bodies. There were a few phagocytes containing small inclusions, and an occasional lymphocyte (see Figs. 1 and 2). Cultures of this specimen of fluid were negative.

The bodies seen in the smear were identified by Dr. Anna Williams, of the Department of Health, as sporotrichia spores.

Within a few days the left eye became blind. The patient had severe headache much of the time, and cerebral vomiting was frequent. Lumbar punctures were done at least once a day to relieve the meningeal irritation symptoms, and from 40 to 60 cc. of fluid was drawn each time with, as a rule, immediate improvement, lessening of the headache, decrease of the drowsiness, and cessation of vomiting. There was a slight fever, the rectal temperature varying between 99° and 101°F.

During the next three weeks no new clinical phenomena appeared, but there seemed to be periods of subsidence of all symptoms, alternating with periods of exacerbation lasting two or three days. With the increase of meningitic signs the spinal-fluid pressure would be greater, there might be a slight fever, and the blood count would show a leukocytosis of from 14,000 to 20,000 cells, the polymorphonuclears ranging from 82 to 90 per cent.

On April 10, routine examination of the fundi showed an unusual picture in the left retina. Previously, the right retina exhibited a mild papilledema and slightly congested vessels, and in the left retina were the same splashes of peripapillary exudate that were first observed on March 6, dilated veins with occasional hemorrhages near the disk, and a nerve-head elevated about 4 diopters. Now to the temporal side of the left optic disk appeared a glistening pearly-white elevation, with borders serrated as are the edges of a cumulus cloud. This mass was 6 diopters in elevation, and across its surface ran small veins and arteries which dipped down at the edges and passed along with the retinal vessels. The retinal vessels were very tortuous, and there were large numbers of small hemorrhages scattered over the entire retina. This pearly-white mass seemed to be utterly unlike what one observes in a detached retina, and was regarded as of inflammatory origin.

With the exception of a period of two and a half weeks in April, the course of the disease seemed to be uninfluenced by any treatment. It was characterized by periods of relapse every seven to ten days, and in the last month by a gradual but steady increase in the signs of a posterior fossa meningitis. As a rule there was no fever, but during the relapses the temperature at times reached 101°F. Death came on June 19, five months and three weeks after the onset of the illness.

From the clinical standpoint there were several important features which must be considered.

From March 5 until the termination of the illness on June 19, a period of three and a half months, there were few new signs indicating extension of the disease process.

Roentgen ray of all the bones of the body showed no evidence of any infection with the sporotrichia. Roentgen ray of the gastro-intestinal tract was negative. The lungs showed a moderate degree of bronchial fibrosis. Cultures of saliva, sputum, naso-pharynx and tonsil smears, urine, blood and feces were negative for sporotrichia.

Roentgen ray of the skull revealed a thinning of the vault associated with a long-continued increase in intracranial pressure.

I. Spinal Fluid. The pressure of the spinal fluid was constantly and markedly increased. On one occasion manometric reading showed a pressure of over 600 mm. water. Lumbar punctures were done daily for therapeutic purposes.

The table indicates the variations in the fluid found on routine examinations. As a rule from 40 to 60 cc. were drawn at each tap. There were only 20 to 30 lymphocytes to the cubic millimeter, although the fluid was usually blood-tinged and contained red cells in amounts from a few hundred to 2000 or 3000 per c.mm. The spinal fluid was as a rule more bloody during the relapses, which would last two or three days.

Following two intraspinal injections of sodium iodid, there was an exacerbation of meningitic signs, and, for a few days, the spinal fluid contained about 5000 white cells per cubic millimeter, of which nearly 100 per cent. were polymorphonuclear cells.

Wassermann and colloidal-gold tests were negative. No tubercle bacilli were found at any time, and guinea-pigs inoculated showed no evidence of tuberculosis.

On standing at room temperature or in the incubator, the fluid would at times become opalescent.This phenomenon was unfamiliar to us and we were unable to determine its cause or chemical nature.

Hanging drop specimens from a large number of samples of spinal fluid were examined. In every specimen were visible small oval bodies which were approximately 2 or 3 micra wide and 3 to 5 micra long. These were refractile, and many of them seemed to contain at the smaller end what might be taken for a polar body. They exhibited a peculiar sort of motion. It was a combination of Brownian movement, with a revolving about the long axis of the body. This motility would decrease during examination and was

TABLE—SPINAL FLUID ANALYSIS

Color and date of appearance.	Lymphocytes per cubic millimeter.	Erythrocytes.	Globulin.	Sugar (per cent.)	Hanging drop.	Stained smears.	Cultures.
March 10. Clear, colorless	2	0	2+	0.068	160 spores	Spores, no other organisms	Negative to 21 days
March 13. Clear, few flocculi	3	0	2+		40 spores	Negative	Negative to 21 days
March 14. Clear, colorless	20	0	2+	0.068	20 spores	Spores	Negative to 21 days
March 15. Hazy, yellowish	8	Many	2+		72 spores	Spores	Negative to 21 days
March 18. Hazy, yellowish	9	A few	2+	0.051	Few spores	Spores	Negative to 21 days
March 19. Clear, colorless	2	0	3+	0.088	Few spores	Spores	Negative to 21 days
March 21. Cloudy, fibrinous sediment.	4600*	A few	4+	0.048	Few rods	Spores	Negative to 21 days
March 23. Yellowish, bloody	Many	Many	4+		Spores and filaments	Spores and filaments	Mold contamination
March 26. Cloudy, blood tinged	25	Many	2+	0.040	Spores and rods	Spores and filaments	Negative to 21 days
March 30. Slightly hazy	....	....	....	....	Spores and rods	LargeGram-plus rods and spores	Negative to 21 days
April 1. Clear, colorless	0	Occasional	1+		Spores	Spores	Negative to 21 days
April 9. Clear, yellowish	20	Few	3+		Spores	Spores	Mold and Staphylococcus contamination
April 14. Hazy, yellowish	30	Many	2+	0.042	Spores	Spores	Negative to 21 days

* Seventy-seven per cent. polymorphonuclears, 15 per cent. lymphocytes, 8 per cent. arachnoid mononuclears.

NOTE.—The spores were found easily in the hanging drop specimens, but with difficulty in smears of sediment or of supernatant portion of centrifuged spinal fluid. We cannot account for this. We neglected to vary the specific gravity of specimens of spinal fluid before centrifuging them.

not found when the spinal fluid had been standing for some time. The motility of these bodies was not due to convection currents, because the bodies would move about the field, whereas red or white cells would remain

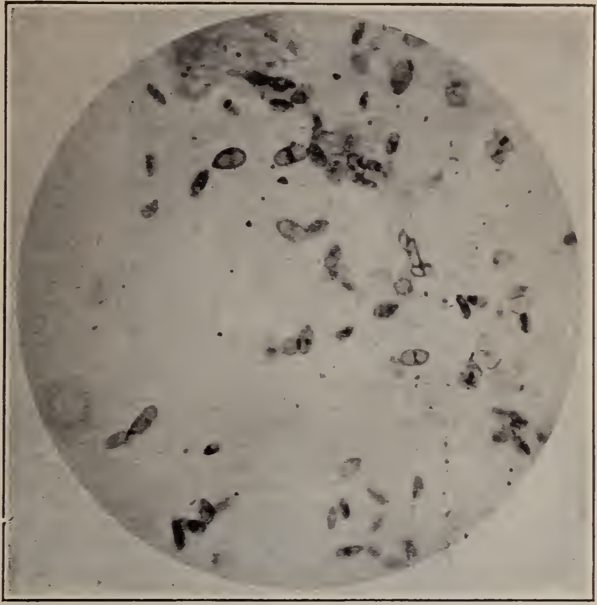

Fig. 1.—Spore forms in spinal fluid smear. (Methylene blue stain.)
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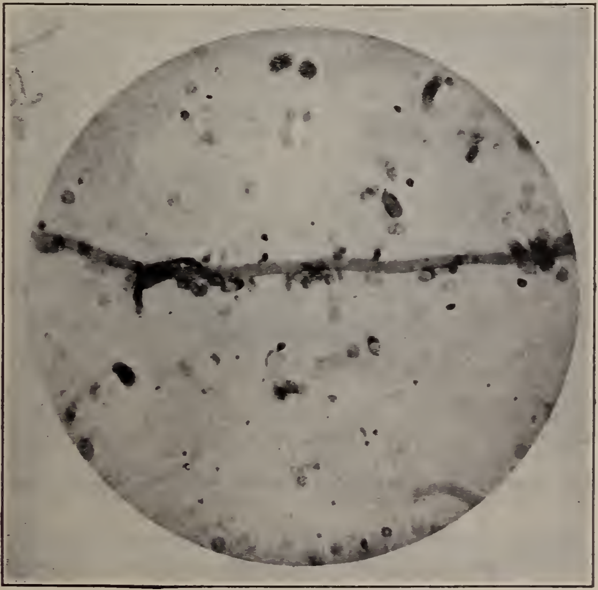

Fig. 2.—Spinal-fluid smear (oil immersion) showing mycelial filament and spores.
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stationary. Occasionally one could see a macrophage, with an ameboid movement, and containing several dark round inclusions. The motile bodies were in a few instances paired end-to-end and once a chain of three was found.

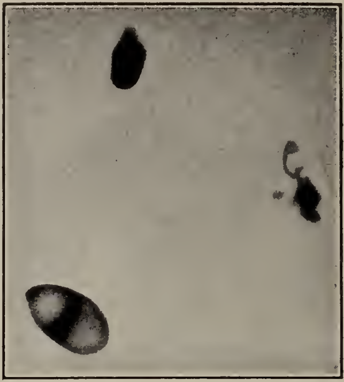

Fig. 2*a*.—Spinal-fluid smear. Oil immersion × 10. Shows dividing form.
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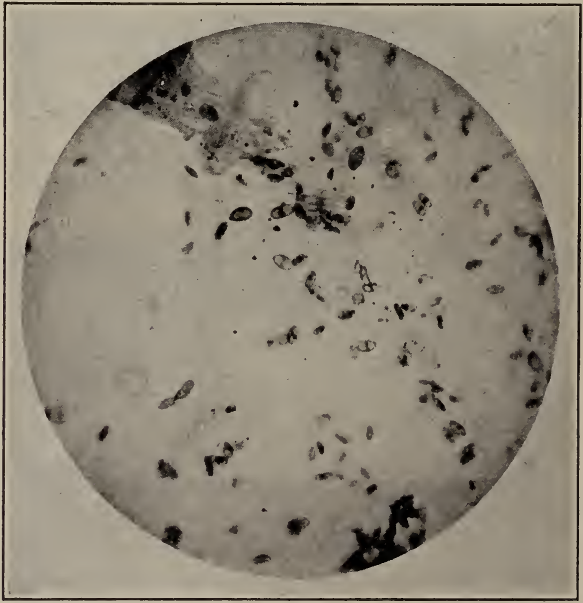

Fig. 2*b*.—Spinal-fluid smear. Shows spores, varying sizes and dividing forms.
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Perhaps half a dozen times rod-like forms with Brownian movement were seen.

Stained smears of the centrifuged sediment of spinal fluid were made. About twenty slides from as many different specimens of spinal fluid were examined (see Figs. 1 and 2). Careful search of each smear revealed in every instance the presence of spore-like bodies. Mycelial forms occurred in two or three of the smears. These bodies stained readily and were Gram amphophilic. Dr. Anna Williams of the Department of Health regarded these bodies as belonging to the pathogenic molds, and stated that they were probably spores of the sporotrichia.

It was observed that these bodies were less numerous in the centrifuged sediment than they were in the hanging drop specimens made from fresh spinal fluid.

Cultures were made from many samples of the spinal fluid, in the laboratory of the Neurological Institute and also in the laboratory of Dr. Neal. Aërobic and anaërobic cultures were made and a large variety of sugar, blood and broth media were used in attempts to grow cultures at both incubator and room temperatures. We failed to secure any positive cultures. Both mold and bacteria-contaminating organisms were occasionally found. It is impossible to explain why these numerous attempts at cultures failed, especially when the sporelike bodies were constantly found in smears made of the centrifuged sediment of spinal fluid.

Dr. Elser (personal communication to one of us), has observed one case of skin sporotrichosis in which positive cultures could not be obtained, and he considered it possible that iodid medication might have affected the organism so that it would not grow in culture.

II. Tetanoid Attacks. From March 16 to May 25 there occurred occasional mild attacks of tingling in the extremities, associated with spasm of the muscles of the hands, which assumed the accoucheur position. From May 25 until death, such attacks occurred almost daily and, when more severe, there was extension of the legs and trismus. Myoclonic movements of the abdominal muscles accompanied these tetanoid attacks during the last few days of life.

III. Temporal Lobe Phenomena. The first occasion on which it seemed that the temporal lobe was involved was during the attack of confusion and aphasia on March 5. This attack and the one on March 10 were the most definite instances. However, in the last month of her illness, the patient frequently would become suddenly confused and lethargic, and for perhaps an hour would exhibit a mild paraphasia.

During two or three of the relapses in which the patient became somewhat disorientated in time and place and became quite forgetful, microptic visual hallucinations were present. These hallucinations were characteristic and were linked in this patient with disturbed function on the left cerebral-hemisphere. The reason for considering the hallucinations as due to a pathological process in the left hemisphere is that the little people appeared consistently in the right half of the visual field. This could be determined not only from questioning the patient, but by observing the patient's behavior when the hallucinations were present.

IV. Treatment. When it seemed that we were dealing with a sporotrichosis infection we began intensive iodid therapy. From about March 15 until May 20, the patient received from 60 to 150 gr. of sodium iodid a day. During March it was given intravenously as well as by mouth; twice it was administered intraspinally. The intraspinal injections caused a great deal of pain, and also produced a rather severe aseptic meningitis. A few injections of salvarsan were given over a period of three weeks in May and early June. Quinin was given in March and discontinued after two or three days because the patient did not tolerate it. It was tried again after the iodids were discontinued and had to be given up for the same reason.

In addition to the above drugs, symptomatic remedies were used. Of these, retention enemas of 25 per cent. salt solution seemed definitely helpful in relieving the vomiting, which was much more frequent during the relapses, and at times when the spinal-fluid pressure was greatest.

Since the sporotrichosis infection produces granulomatous lesions, since we were dealing with a very serious situation, and since it has been observed that intravenous administration of a solution of active deposit or radium emanation has at times had therapeutic effect in Hodgkin's Disease, we felt justified in trying an experiment.

On April 2 the patient was given intravenously 70 millicuries of active deposit solution of radium emanation. At the time this was given the patient had been moderately deaf for about two weeks, so that whispers could not be heard and the patient was unable to hear the children singing in a nearby school or detect the sounds of people passing through the corridor by her room. She had a bilateral facial weakness, there was right internal strabismus, and the signs of meningeal irritation had been troublesome for several days.

The injection was given at 1:30 p.m. At 3 p.m. the patient vomited twice. At 4 p.m. tenderness and swelling of both parotid glands appeared. By 6 p.m. the swelling had extended to the submaxillary glands and also involved the lateral and posterior cervical lymph nodes. The patient was also quite drowsy and complained bitterly of a stiff neck. During the night she observed to the nurse that she was again able to hear sounds which she had been unable to hear for a week. The next morning the meningeal signs were markedly reduced and less than they had been for some days. During the next few days the salivary glands and lymph node swelling disappeared, the eye movements were better, the facial weakness improved, and the spinal fluid gradually became free of red blood cells and the sporelike bodies became scanty in numbers. From about April 10 to 30, the patient's general condition steadily improved and the cranial nerve signs also lessened. The patient became able to walk about the room, stay up most of the day, and even took a few rides about the town in an automobile.

During May when the patient had become worse, another dose of active deposit was given. The same swelling of the salivary glands ensued, and there was also a mild systemic reaction with a temporary exacerbation of the meningeal signs. However, this injection was not followed by the clinical improvement which occurred after the first injection.

It is interesting to speculate upon the phenomena following the first injection. In particular, the swelling of the salivary glands was noteworthy. This complication has not been observed in a series of several hundred intravenous injections of radium deposit at the Memorial Hospital in New York. It is conceivable that the glands became swollen because they were the avenue of entrance of the sporotrichia infection, and, therefore, were already predisposed to the reaction which would be a consequence of the radium attacking infected tissues.

It is also conceivable that the radium, in producing a systemic reaction, upset the vegetative function and caused a temporary disturbance in the gonads. The injured gonads may have then caused a selective sympathetic involvement of the salivary glands, exhibiting in the reverse fashion the relation ship between the salivary glands and the gonads, well known in mumps.

Necropsy. A complete necropsy was done six hours after death. Examination of the viscera showed no abnormality other than might be found due to agonal causes, and nothing which would throw light on the lesions in the central nervous system. The skin showed no lesions of any description, and the mucus membranes were normal with the exception of a mucopurulent conjunctivitis in both eyes. This appeared a few days before death.

The gross pathology was limited to the intracranial cavity. Protocol of the examination of the head and intracranial cavity follows:

Head. Symmetrical and not enlarged. Scalp and pericranium negative. Dura slightly adherent to calvarium; latter thin over vertex. Sutures quite visible. Superior longitudinal sinus empty. Dura everywhere appears normal.

Brain. No bulging. Cerebral hemispheres apparently symmetrical. Veins moderately prominent, but there is no marked convolutional capillary congestion. Convolutions over vertex somewhat flattened and sulci compressed. Pacchionian bodies small. Piaärachnoid over parieto-temporal regions semiopaque and slightly thickened. Cerebellar convolutions fairly well obliterated, particularly on the left side. There is considerable cloudy, bloody fluid in the subtentorial space. Base of brain shows widespread subarachnoid accumulation of cloudy fluid—well marked between the cerebellar hemispheres, where a gelatinous mass 5 by 1½ by 1 cm., is seen The piaärachnoid appears thickened over the inferior surface of the cerebellum and in the interpeduncular space. An occasional small area of hemorrhage is seen in the leptomeninges over the base. No granulomatous tissue, abscess formation, or softening of the cortex observed. There is a gelatinous exudate about the left optic nerve, and in the left orbit, where there is also considerable edema of the orbital fat. Retinæ were not removed.

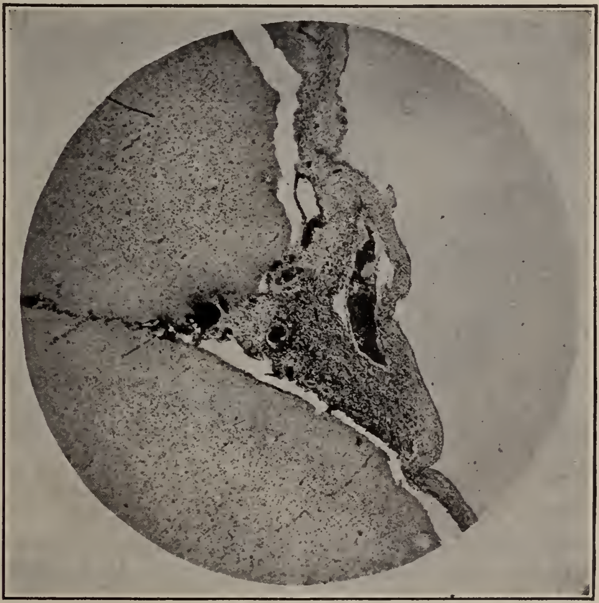

Fig. 3.—Showing leptomeningitis, temporo-parietal cortex. (Hematoxylin-eosin stain).

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There is slight internal hydrocephalus as determined by puncture of the ventricles, 10 cc. being withdrawn from the left and 5 cc. from the right. Fluid slightly hazy.

The ethmoid, sphenoid, frontal and mastoid sinuses and the middle ears were opened and were all found to be perfectly normal and free of any fluid, mucus, or inflammatory thickening of the membranes.

On section, after hardening in 10 per cent. formalin, the brain showed softening of both basal ganglia, probably postmortem. There was a very marked thickening of the piaärachnoid and of the ependymal lining of the ventricles. The spinal cord was not removed.

Anatomic Diagnosis. Chronic diffuse leptomeningitis, with gelatinous exudate beneath piaärachnoid. Exudative periophthalmitis and perineuritis (left).

Histologic Examination. [Stains: Hematoxylin and eosin, Mallory, Nissl, van Gieson and Loyez (myelin)].

Temporo-parietal Area. There is a marked thickening of the pia-arachnoid with increase of connective tissue and great infiltration of the pia with numerous lymphocytic and plasma cells (Fig. 3). In the cortex there is well-marked perivascular edema with occasional scattered round cells in the Virchow-Robin spaces (Fig. 4). There is edema of moderate degree in the outer layers of the cortex, but no hemorrhages. No degenerative changes of ganglion cells beyond scattered very slight swelling of isolated cells in the outer layers of the cortex. No glia proliferation. In the subcortical region there are numerous intraglial foci filled with metachromatic lipoids, but there is no degeneration of the myelin sheaths.

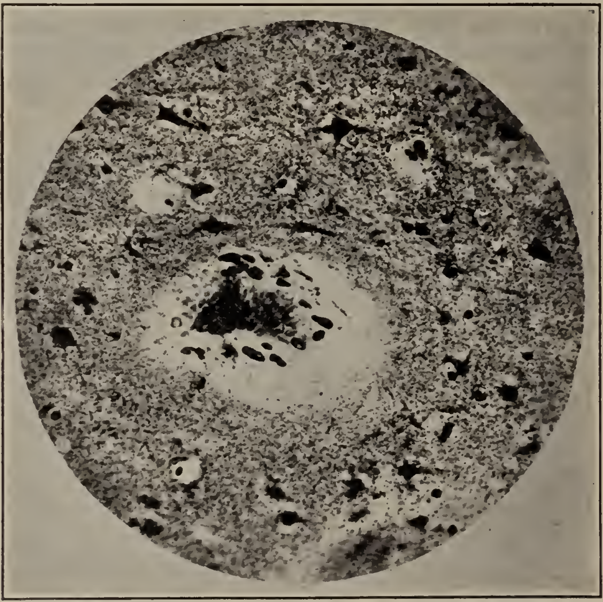

Fig. 4.—Perivascular infiltration of temporo-parietal cortex. (Rio Hortega stain.)

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Cerebellum. The picture of the meninges covering the cerebellum is similar to that described (cerebral). The perivascular edema within the cerebellum is of minor degree with absence of cellular infiltration. Foci of the mycelium forms with spore inclusions were found in the meninges covering the cerebellum.

Medulla. The leptomeningitis is also found surrounding the medulla oblongata. The Loyez (myelin) stain shows swelling of the myelin sheaths throughout the whole section, most pronounced at the periphery where transitions to partial myelin sheath degeneration and complete loss of myelin sheaths are noticed.

HISTOLOGIC DIAGNOSIS. Severe chronic leptomeningitis and low-grade cerebral edema.

ANIMAL INOCULATIONS. The animal inoculations were carried out in the laboratory of Dr. Josephine B. Neal. The results follow:

A. FROM SPINAL FLUID OF PATIENT. *Mice.* March 19. Two mice injected intraperitoneally; mice living three months later.

March 22. Three mice injected intraperitoneally. One died of pneumonia, one died of brain hemorrhage and one died of causes unknown.

March 29. Three mice injected intraperitoneally. One mouse living, two mice on necropsy two weeks later had tapeworm and also showed enlarged and reddish lymph nodes, adherent viscera and deposits of gelatinous substance in the peritoneum. Cultures negative.

April 14. Nine mice injected intraperitoneally; living four months later.

Guinea Pigs. March 19. Two guinea-pigs injected intraperitoneally. Both died of pneumonia and cultures showed pneumonococci.

March 20. Two guinea-pigs injected intraperitoneally. One of the guinea-pigs died of tuberculosis.

March 22. Two guinea-pigs injected intraperitoneally. One guinea-pig showed, on necropsy, enlarged inguinal and axillary lymph nodes, and peritoneal and pleural fluid; cultures were negative.. The second guinea pig on necropsy, showed mottled lungs, normal kidneys and liver, and gelatinous masses in the peritoneal cavity. Cultures were negative.

June 9. Two guinea pigs injected intracranially. One died in twenty-four hours presumably of shock; the other guinea pig is alive.

Rabbits. June 9. Two rabbits injected intracranially. Both rabbits died of septicemia which was passed to subculture guinea pigs. The cultures showed cocci and bacilli.

B. VENTRICULAR FLUID (POSTMORTEM ON PATIENT). June 19. Three guinea pigs injected intraperitoneally. One guinea pig is alive. Another guinea pig died July 11; the brain was found to be congested. Cultures negative. The third guinea pig died July 8 and showed congested brain and lungs; cultures were negative.

Subcultures on two guinea pigs from this third animal gave negative results.

C. EMULSION OF BRAIN SWABS OF GELATINOUS EXUDATE (FROM BRAIN OF PATIENT). June 19. Four rabbits injected intracranially. Two died of injection shock; one died of septicemia, which was passed to guinea pigs; one at death showed hemorrhage of the brain, cultures of which were negative.

June 19. Three guinea pigs injected intraperitoneally. One is alive, another guinea pig died in September of pneumonia, and a third died three days after inoculation. Necropsy was not done.

D. RÉSUMÉ OF EXPERIMENTAL WORK. Six rabbits, 14 guinea pigs and 17 mice were inoculated. Of these, 2 mice and 1 guinea pig, which were inoculated intraperitoneally with spinal fluid from the patient, showed a gelatinous exudate in the peritoneal cavity. This exudate was, in appearance, similar to that found under the meninges of the patient.

Inoculations of postmortem material from the patient gave results which were not helpful.

Examination of the gelatinous exudate found in the mice and guinea pigs revealed no organism.

Histologic study of postmortem material of inoculated animals was not helpful and corroborated the clinical diagnosis as to cause of death only.

Summary. Sporotrichosis infection of the nervous system has not been observed previously.

Our patient was a girl, aged fifteen years, who became ill December 30, 1924, and died June 19, 1925. The infection involved the posterior fossa largely, had a febrile onset, and ran a chronic remitting course. There were slight exacerbations about every ten days, accompanied by a slight fever.

Interesting clinical occurrences were tetanoid attacks, microptic hallucinations, and several temporosphenoidal seizures.

The diagnosis was made by the presence of sporotrichia spores and mycelial forms in the spinal fluid.

Iodid therapy was of no avail. Experiments with radium intravenously gave interesting results.

Culture and animal inoculation results were negative.

Microphotographs of the spores seen in the spinal fluid and of the tissue from the patient's brain are exhibited.

Autopsy of the patient showed a chronic diffuse leptomeningitis most marked in the posterior fossa, a moderate edema of the brain and the presence of a peculiar gelatinous exudate here and there beneath the pia. Microscopically, there was shown a chronic productive leptomeningitis but no inflammatory involvement of the brain substance.

For bibliography see the original article.

STREPTOCOCCIC MENINGITIS FOLLOWING SCARLET FEVER—RECOVERY

Published in The Archives of Pediatrics, 1927, Vol. XLIV, pp. 395-398

By Josephine B. Neal and Archbold Jones

The mortality in streptococcic meningitis is so high that it is a matter of interest when a patient suffering from it recovers. This case is one of particular interest because there is reasonable ground for believing that the treatment was specific.

A. P., a girl 7 years of age, was seen by Dr. Emanuel Appelbaum of the Meningitis Division of the New York City Health Department on January 30, 1927. There was a history of a mild attack of scarlet fever beginning on January 20, 1927. The symptoms lasted only two days and the following two days she seemed perfectly well. The meningeal symptoms then developed suddenly with fever and headache. Her condition became decidedly worse on the morning of January 30.

On physical examination many of the typical signs of meningitis were present. There was marked stiffness of the neck and a positive Kernig and Brudzinski sign. The pupils were equal and reacted to light. The fundi of the eyes were normal. The patella reflexes were absent; the biceps and abdominals were diminished. There was a Babinski sign on the left but the confirmatory signs were absent on both sides. There was a left otitis media. She was clear mentally. The heart, lungs and abdomen were normal. The temperature was 103°F.; pulse, 100; respiration, 20. On lumbar puncture, 60 cc. of purulent fluid, under greatly increased pressure, was withdrawn and 20 cc. of anti-meningitis serum was injected, although the history of scarlet fever and an otitis media suggested the strong possibility of a streptococcic meningitis. The examination of the spinal fluid showed a very great increase in cells, practically all polymorphonuclear. There was a moderate increase in protein and an absence of sugar. No definite organisms were demonstrated in the smear but the culture showed a hemolytic streptococcus.

The patient was admitted to Willard Parker Hospital late at night on January 30, presenting much the same clinical picture. On account of the history of scarlet fever and the presence of desquamation, 30 cc. of antiscarlatinal serum No. 14 was given intramuscularly. This is a combined antitoxic and antibacterial serum prepared under the direction of Dr. W. H. Park and Dr. A. W. Williams and described by them as "Antiscarlatinal Serum of Dual Potency." (Proceedings of Soc. of Exp., Biol. and Med., 23; 2839, 1925).

On the morning of January 31 a lumbar puncture was performed at the Hospital and, as it was not known that the previous fluid had been cultured, antimeningitis serum was given again. This spinal fluid showed Gram positive cocci by smear and a hemolytic streptococcus by culture. Later that same day another puncture was done and about 15 cc. of scarlatinal serum No. 14, containing ¾ cc. of one per cent. neutral acriflavine, was injected. At this puncture only 2 or 3 cc. of spinal fluid could be obtained, indicating that there was perhaps some block developing. The serum ran in with some difficulty. The child seemed to be desperately ill. She was semi-delirious and the temperature was between 104°F. and 105°F.

On February 1 and 2 the condition was about the same. The neck was greatly retracted. Only 1 or 2 cc. of blood-tinged fluid could be obtained by lumbar puncture but 15 cc. of the antiscalartinal serum was injected in part by gentle pressure along the tube of the gravity apparatus. This indicated a block rather high up and the use of the dye was discontinued after the first injection, for it was feared it might increase the tendency to the formation of adhesions. These spinal fluids were sterile.

On February 3 a cistern puncture was easily done, 45 cc. of cloudy fluid being removed and 20 cc. of antiscarlatinal serum being injected. This fluid gave a culture of hemolytic streptococcus, although no organisms were demonstrated in the smear. The protein was somewhat less than in the first two fluids and the sugar was normal, by the Fehling's reaction.

On February 4, a cistern puncture was unsuccessful, due, it was felt, to adhesions, as the needle seemed to penetrate the dura. About 15 cc. of the antiscarlatinal serum was given intraspinally, although but a few drops of blood-tinged fluid could be obtained.

On the 5th and 6th fifteen cc. of the serum were given, only a few drops of fluid being obtained each time.

On the 7th another unsuccessful attempt at cistern puncture was made, but a lumbar puncture, while the child was still under the anesthetic, yielded 8 cc. of yellowish, slightly cloudy fluid, and 15 cc. of serum was injected. This fluid was negative for organisms by smear and sterile by culture. The protein was somewhat higher than in the cisternal fluid. The sugar was normal by Fehling's. All this time the temperature had been running between 101° and 104° to 105°. Much of the time she had been irrational. The left ear had drained rather freely at times but there had been no indication of mastoid involvement, either clinically or by the roentgen ray examination. An examination of the eye grounds on February 1 showed some blurring of both discs but no peripapillary edema or chocking of the discs.

On February 8, the child seemed distinctly better. The neck was less retracted. She was rational and the temperature was lower—99.5° to 102.4°.

On February 11, an extensive serum rash developed, which was probably a factor in keeping up the temperature, which did not become normal until February 18. On the 11th a slight internal strabismus of the right eye was noted. This gradually disappeared.

From this time on her convalescence was fairly satisfactory. There were two exacerbations of fever, probably due to the lighting up of a pyelitis from which she had suffered some five years before and which had troubled her at intervals ever since.

Pus and albumin were found in the urine during one of these periods. Six other examinations of the urine were negative.

A blood count on February 2 showed 22,600 white blood cells, 83 per cent. polymorphonuclears, 11 per cent. lymphocytes, 6 per cent. mononuclears. Two blood cultures, one on the 2nd and one on the 20th of February, were negative.

The culture of hemolytic streptococcus from the spinal fluid was given to Dr. Anna W. Williams, who reported it as belonging to the chief scarlet fever group. It completely absorbed the antibody in serum produced by scarlet fever strain No. 55.

This identification of the causative organism seemed good reason for believing that the serum used was the specific factor in the recovery. The administration of the serum into the cistern was probably of great value, especially since the fluid withdrawn still contained living organisms. The development of block always renders the prognosis of a meningitis case much more serious. The only clinical features that could be referred to the adhesion were some loss of control of the sphincters and spasticity of the legs, more marked on the right side.

These manifestations gradually disappeared. The patient returned home in excellent condition on March 13, 1927. Her progress since that time has been entirely satisfactory.

Streptococci meningitis following scarlet fever is comparatively rare. We have a record of only four other cases. In all of these there was also an otitis media and in two of them a mastoid operation was performed. All resulted fatally. In this particular case it seems impossible to say whether the meningitis was secondary to the otitis media or whether both conditions developed more or less simultaneously and independently.

The high mortality of streptococci meningitis is shown by the fact that this is only the third case to recover among the 116 cases that have been seen by the Meningitis Division.

SPONTANEOUS MENINGEAL HEMORRHAGE

Published in The Journal of the American Medical Association, 1926,
Vol. LXXXVI, pp. 6-8

Josephine B. Neal

While the French have developed an extensive literature on spontaneous or medical meningeal hemorrhage, comparatively little has been written on the subject by the physicians of other nations. Aside from an article by Leopold, a chapter that the writer contributed to Tice's Practice of Medicine and a case reported by Dawson, there are but brief references to the subject in English. In Sweden, two comprehensive articles have been published by Ehrenberg and Forsheim, respectively. Only a few articles have been written on the subject in Germany. No attempt will be made to review the literature, the principal references only being given.

During the fifteen years that the members of the meningitis division of the Health Department of New York City have been studying acute conditions of the central nervous system, especially meningitis, poliomyelitis and epidemic encephalitis, we have seen over thirty cases, occurring for the most part in children and young adults, in which a diagnosis of meningeal hemorrhage was made. So few of the physicians with whom we have seen these cases have been familiar with the condition that it seems worth while to describe some of the more interesting cases in detail and to present certain data in regard to the others. These are listed in the accompanying table.

The early symptoms of the cases, especially when there was no underlying disease, were those of an acute, often of a fulminating, meningitis. The onset was sudden, with intense headache, usually vomiting, and not infrequently unconsciousness within a few hours. In some instances, there were twitchings or convulsions. Stiffness of the neck, a positive Kernig sign and various changes in the reflexes occurred within a few hours to a day or two. In most instances, there was a moderate elevation of temperature (from 1 to 3 degrees.) The pulse was usually, but not invariably, slow, especially early in the case. Ehrenberg, followed by Leopold, divides the cases into two types, meningitic and apoplectic. This has seemed unnecessary, as merely emphasizing a greater or less severity of onset which might easily be further subdivided. It has not seemed to influence the outcome, as several patients with early unconsciousness have made complete and, in some instances, fairly rapid recoveries.

In this group of cases none have been included except those noted below that showed spastic paralysis, as of course this would have led to the diagnosis of cerebral hemorrhage. In several instances there was a slight and transitory weakness of the muscles of the face or hand, probably due to pressure. Two cases with more marked paralysis came to necropsy and showed no evidence of cerebral hemorrhage.

Guillain states that the symptoms are due both to pressure and to the toxic products of hemolysis. The diagnosis has been made chiefly by the spinal fluids, which have contained varying amounts of blood in the different cases. The blood at a given puncture has been evenly distributed through the fluid, as shown by collection in successive tubes, and it has not coagulated. When the lumbar puncture was done very soon after the onset of symptoms, the supernatant fluid, on centrifugalization, was nearly clear, or slightly reddish or yellowish. In later fluids, the supernatant liquid was yellow, the depth of color depending on the amount of the hemorrhage. The spinal fluids have been sterile on culture, except in three instances in which the meningeal hemorrhage accompanied an epidemic meningitis. In many instances the Wassermann test has been done on the spinal fluid and has been negative (except in Case 22 in the table). The cytologic and chemical examination have not been of much value in most of the cases on account of the blood.

The following case history is fairly typical:

Case 11.—M. W., a girl aged 12 years, whose past history was negative except for measles, was seen at midnight, Aug. 10, 1917. During the day she had received a slight blow on the head while playing with her brother. She had been taken suddenly ill at 2 p. m. with severe headache and vomiting, and began having periods of delirium. At midnight she was semistuporous; the pupils reacted sluggishly to light; the patellar reflexes, which had been exaggerated earlier, were nearly lost; there was stiffness of the neck and a positive Kernig sign; the temperature was normal, and the pulse 77 and slightly irregular. At lumbar puncture, 30 cc. of uniformly bloody fluid was withdrawn, and since the picture so strongly suggested meningitis and since horse serum is indicated in hemorrhage, 20 cc. of antimeningitis serum was given. On the following day the condition was slightly improved. The patient was less stuporous and more rational. At lumbar puncture 30 cc. of somewhat less hemorrhagic fluid was withdrawn and 20 cc. of serum again administered. On the 12th and 13th, no punctures were done, as the condition seemed to be improving. On the 13th she began to have a rather low, irregular fever. August 14 thirty cc. of bloody fluid was withdrawn but no serum was given, as the cultures from the previous fluids were sterile. The Wassermann test on the spinal fluid was negative. On the 15th another puncture was done, as the meningeal symptoms remained marked. The patient was sent to Bellevue Hospital, August 16, where she remained until the middle of October. The meningeal symptoms persisted for some time and necessitated repeated lumbar punctures, the earlier of which revealed hemorrhagic fluids. She complained of rather frequent headaches for some months after she left the hospital. When last seen, in the fall of 1924, seven years later, she showed no evidence of having suffered any ill effects from the meningeal hemorrhage.

A similar but much milder case is the following:

Case 8.—G. K., a boy aged 9 years, with no history of a previous illness, was taken suddenly ill at 2 a. m., April 29, 1918, with headache, vomiting, and occasional periods of delirium. When examined, May 3, he showed moderate stiffness of the neck, marked Kernig sign, absence of the patellar reflexes, and contracted pupils. He was no longer delirious but was slightly stuporous. His pulse was 86 and irregular. He had had a low irregular fever. At lumbar puncture 15 cc. of hemorrhagic fluid was obtained and an equal amount of meningitis serum given, for the reasons already stated. The culture from the fluid was sterile and the Wassermann reaction negative. The boy made a rapid and uneventful recovery, and more than six years later was reported to be entirely well.

The following case was seen at the Jamaica Hospital through the kindness of Dr. E. E. Keet:

Case 18.—J. S., a youth aged 19, had suffered from a severe electric shock a year previous to his illness and since that time had had frequent intense headaches, slight haziness of vision, twitching of the left hand, and slight left facial weakness. He was taken ill suddenly, Jan. 24, 1925, with headache, vomiting and early unconsciousness. He was seen, January 28. At this time there was moderate stiffness of the neck and Kernig sign. There was no limitation of motion of the eyes, no ptosis and no strabismus. The pupils reacted normally to light and in accommodation, and the fundi were normal. There was some facial weakness, more marked on the right. The patellar reflexes were diminished; there was no ankle clonus or Babinski sign, though the latter had been present on admission. The abdominal reflexes responded irregularly at different examinations. There was a rather generalized muscular tremor at times. The patient was still semistuporous. His speech was somewhat blurred. He had been having an irregular fever, up to 102. His pulse had varied from 50 to 90 but was regular in rhythm. The systolic blood pressure was 130; diastolic, 70. The blood count showed a moderate leukocytosis, 16,000. There was incontinence of urine. The lumbar punctures had yielded uniformly blood-tinged fluid. The Wassermann reaction on the spinal fluid was negative. The patient remained in the hospital for almost a month, gaining steadily. On leaving the hospital there was some weakness of the left hand, but this disappeared in about a month. The diagnosis of epidemic encephalitis was considered in this case, but meningeal hemorrhage was favored on account of the suddenness and severity of the onset. Moreover, hemorrhagic fluids are rare in epidemic encephalitis.

The following case was seen at Bellevue Hospital through the courtesy of Dr. Charles E. Nammack:

Case 25.—D. S., a woman aged 24, with a history of good general health, was not subject to headache until the two weeks preceding the illness. When the illness began, Oct. 4, 1924, she had been riding in a taxicab and had struck her head against the side of the cab, but there was no contusion of the scalp, and the roentgenogram of the skull showed no evidence of fracture. She became suddenly ill with epistaxis, vomiting, headache and pain in the back of the neck, and speedily became unconscious. The patient was examined October 7. At that time she was still quite stuporous; there was moderate stiffness of the neck and Kernig sign. The pupils were equal and reacted to light and in accommodation; there was bilateral ptosis and weakness of the left external rectus. The disks were choked. There was also slight facial weakness. The patellar reflex was increased on the left and diminished on the right. There was no ankle clonus. There was a positive Babinski sign on the left. There was no sensory disturbance. A low irregular fever ranged from 100 to 101°F. The pulse rate was from 60 to 70. The blood pressure was 120, systolic, and 80, diastolic. The blood count showed a low leukocytosis, 13,600; polymorphonuclears, 81 per cent. The urine was not abnormal. Chemical examination of the blood revealed: blood sugar, 140; nonprotein nitrogen, 34; sodium chlorid, 495. The blood and spinal fluid Wasserman reactions were negative. The spinal fluid was hemorrhagic. On account of the choking of the disks, a diagnosis of brain tumor was considered. However, the choking of the disks disappeared on repeated lumbar puncture, as did also the weakness of the face and eye muscles. The patient left the hospital greatly improved, and subsequent examinations have shown her to be entirely well after several months.

Case 10.—J. M., a girl aged 12, seen June 5, 1916, had had measles and scarlet fever some years before, and there was a history of an indefinite illness two months previously, with pain and swelling of the legs, and fever. She had been taken ill while at a dance late on the night of June 4, with headache and vomiting, followed very quickly by unconsciousness. June 5 she was semistuporous. There was stiffness of the neck and a positive Kernig sign. The patellar reflexes were absent; the pupils were unequal. The pulse was 84 and regular and the temperature, 100°F. At lumbar puncture, 35 cc. of hemorrhagic fluid was obtained, and 20 cc. of antimeningitis serum given. June 6 the patient was taken to Mount Sinai Hospital, where she died three days later.

Necropsy (by Dr. F .S. Mandelbaum).—The body was that of a poorly nourished young girl, showing marked rigor mortis and postmortem discoloration. There were no marks of injury on the body.

The skull was of normal thickness. The surface of the dura was normal in appearance. All the cranial blood sinuses were opened and found normal. Sphenoidal sinuses and both middle ears were opened and found normal. The internal surface of the dura was normal in appearance. The cerebrospinal fluid was normal in amount but, as it was mixed with blood from the necropsy, its characteristics could not be noted.

The brain was intensely congested and extremely bright red. Over its convexity were numerous small, bright red petechial hemorrhages. In the pia-arachnoid, at the base of the brain, especially over the pons, were great numbers of bright red, hemorrhagic areas from 2 to 6 mm. in size. The blood vessels were normal and contained fluid blood, but even the minute capillaries were congested and visible. Over the surface of the brain were two or three pale, miliary areas which had somewhat the appearance of tubercles. The brain was uniform in appearance and consistency. An attempt was made to aspirate the ventricles, but was unsuccessful. On section, the brain showed, throughout, the same extreme congestion noted on the surface; all the ventricles were normal, and the brain was otherwise normal.

Cultures from the brain showed *B. coli*. The smears were negative for tuberculosis. An inoculation, July 20, 1916, was negative for tuberculosis.

Smears with Gram's stain and diluted carbolfuchsin showed a moderate number of polymorphonuclears, but no organisms.

Microscopic examination of two sections of the cortex revealed a marked edema of the pia, and also a diffuse infiltration with red blood cells and few polymorphonuclears. There was a marked congestion of all the blood vessels of the pia and the brain. A moderate number of very small hemorrhages were found in the cortex. The hypophysis showed extreme congestion of the blood vessels and a small amount of colloid in some of the alveolar spaces in the pars intermedia and pars anterior. Acidophilic cells were very prominent and were present in great numbers; otherwise, the section was normal.

The Giemsa stain showed no bacteria.

In the cervical cord and pons there was congestion of the blood vessels and the same type of inflammation of the pia-arachnoid as noted in the meninges of the cerebrum. No evidence of poliomyelitis was found. The diagnosis was acute encephalitis.

It will be noted that it was assumed at the necropsy that the blood in the spinal fluid was accidental. While a diagnosis of acute encephalitis was made, it is evident from a study of the report that the changes were more marked in the meninges than in the brain substance.

Cases 13, 26 and 28 were of interest:

Case 13.—A girl aged 15 years, gave a history of a similar illness nine years before, which had lasted about three weeks. She had had another mild attack in 1915, and made an uneventful recovery. Two lumbar punctures were done, yielding 50 and 60 cc. respectively, of slightly hemorrhagic fluid.

Case 26.—A man aged 27, gave a history of a similar illness, not so severe, four years previously. His attack in 1918 was a severe one, with unconsciousness at the onset. The fluid was hemorrhagic at several punctures.

He made an apparently complete recovery but died in a similar attack some years later.

CASE 28.—A man aged 30, seen in June, 1915, had had a similar attack eighteen years before, from which he had recovered completely, and a second attack five years before, which was followed by some mental impairment. The attack in 1915 was only moderately severe, and he recovered so far as his physical condition was concerned, but the mental impairment had apparently increased.

DATA IN THIRTY-FIVE CASES OF MENINGEAL HEMORRHAGE

No.	Year	Age	Sex*	Possible Cause	Outcome	Comment
1	1923	2 mo.	♀	Not determined	Died	
2	1917	5 mo.	♀	Epidemic meningitis	Died	
3	1918	9 mo.	♀	Not determined	Died	Liver enlarged; jaundice
4	1912	22 mo.	♂	Epidemic meningitis	Recovered	
5	1925	4 yr.	♀	Mitral lesion and Streptococcus viridans septicemia	Died	
6	1916	5 yr.	♀	Epidemic meningitis	Died	
7	1923	7½ yr.	♂	Hydrocephalus; mitral regurgitation	Died	
8	1918	9 yr.	♂	Not determined	Recovered	
9	1923	10 yr.	♀	Mitral stenosis	Recovered	
10	1916	12 yr.	♀	Not determined	Died	Necropsy finding reported
11	1917	12 yr.	♀	Not determined	Recovered	
12	1917	14 yr.	♂	Nephritis	Recovered	Died a month later with nephritis
13	1915	15 yr.	♀	Not determined	Recovered	History of similar illness 9 years before
14	1918	16 yr.	♂	Pneumonia	Recovered	
15	1919	17 yr.	♀	Not determined	Died	
16	1923	18 yr.	♂	Not determined	Died	Necropsy; pachymeningeal internal hemorrhage
17	1917	19 yr.	♀	Not determined	Recovered	
18	1925	19 yr.	♂	Not determined	Recovered	Severe electric shock year ago
19	1914	20 yr.	♂	Not determined	Died	
20	1924	20 yr.	♀	Not determined	Recovered	History of epidemic meningitis 10 years ago
21	1918	20 yr.	♀	Epidemic meningitis	Recovered	
22	1917	21 yr.	♂	Syphilis	Recovered	Sent to State Hospital
23	1915	22 yr.	♂	Blood pressure, 190 systolic	Died	No focal symptoms pointing to cerebral hemorrhage
24	1914	22 yr.	♀	Endocarditis	Died	
25	1924	24 yr.	♀	Struck head in taxicab, but no external mark	Recovered	

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* In this column, ♂ indicates male; ♀, female.

DATA IN THIRTY-FIVE CASES OF MENINGEAL HEMORRHAGE—*Continued*

No.	Year	Age	Sex*	Possible Cause	Outcome	Comment
26	1918	27 yr.	♂	Not determined	Recovered	History of previous attack 4 years ago; died in similar attack some years later
27	1924	27 yr.	♂	Not determined	Recovered	
28	1915	30 yr	♂	Not determined	Recovered	History of 2 previous attacks; mental deterioration after second and third
29	1917	34 yr.	♀	Not determined	Recovered	
30	1919	36 yr.	♂	Not determined	Recovered	
31	1925	36 yr.	♀	Not determined	Died	Necropsy showed hemorrhage in right ventricle
32	1920	40 yr.	♀	Not determined	Recovered	
33	1923	46 yr.	♂	Not determined	Recovered	
34	1923	48 yr.	♂	Not determined	Died	Necropsy showed hemorrhage in left ventricle and around circle of Willis
35	1923	50 yr.	♀	Not determined	Recovered	

* In this column, ♂ indicates male; ♀, female.

While realizing that it is possible that these other attacks were different in origin, it seems reasonable to assume that they were of the same nature. If they were, the possibility suggests itself that they represented stages in the development of pachymeningitis interna hemorrhagica. It will be recalled that, on necropsy in this condition, there are often several layers in the false membrane beneath the dura, showing successive episodes in its formation.

It will be noted in the table that in four cases the meningeal hemorrhage accompanied an attack of epidemic meningitis; in four cases, a cardiac lesion; in one, pneumonia; in one, syphilis; in one, nephritis, and in one, high blood pressure. In the great majority of cases, however, no cause could be determined. Froin gives a long list of conditions that may be the underlying causes of spontaneous meningeal hemorrhages. The author stated in Tice's Practice of Medicine: "When we see so many conditions given as the cause of an occurrence, and when we remember that these conditions exist in the vast majority of cases without this occurrence taking place, it seems evident that the real cause must lie elsewhere, perhaps in some inherent weakness of the blood vessel in the individual cases in which meningeal hemorrhage occurs." Meylahn concludes that spontaneous meningeal hemorrhage is nearly always a diapedesis. The causes of such an occurrence are disorders in the function of the vascular nerve apparatus. These vasomotor disturbances can be the result of diseases of the blood vessel walls. In that case, however, there must be assumed a previously existing constitutional weakness of the vascular nerves.

Weil, Isch-Wall and Pollet call attention to the existence of blood lesions. analogous to those of hemorrhagic conditions, existing in certain patients with meningeal or cerebral hemorrhage. There is an increased bleeding time; coagulation is retarded, and the clot is less retractile and easily dissolves. The importance of this from a therapeutic standpoint is obvious.

The most important measure in treatment is lumbar puncture. Guillain warns against withdrawing too much fluid early in the illness. The lumbar puncture should be repeated daily, or less often, depending on the clinical symptoms. In most of our cases it was followed by improvement. In many of our cases horse serum, in the form of antimeningitis serum, was injected intraspinally early. This may have had a favorable influence on the hemorrhage. Certainly when the bleeding and coagulation time are increased, measures should be taken to correct these conditions of the blood.

It will be seen from a study of the table that twenty-one of our thirty-five patients recovered from the meningeal hemorrhage, although one died of nephritis a month later.

Meningeal hemorrhage is certainly a most interesting condition and one that warrants much further study.

For bibliography see the original article.

EXPERIMENTAL POLIOMYELITIS

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Mary Nevin and Florence R. Bittman

The object of this work was to repeat certain experiments on poliomyelitis in rabbits. A number of workers have reported varying results. Some have found it difficult or impossible to inoculate rabbits and produce a picture resembling poliomyelitis; others assert that they were able to make successful inoculations and to pass the virus through a number of generations. All who were successful have found, however, that it was only in young rabbits that any effects were produced; that the results were variable and that the symptoms were always different from those observed in monkeys and in man.

The most recent work on experimental production of poliomyelitis in rabbits is that of Rosenau and Havens. They passed poliomyelitis virus from a monkey through eight generations of rabbits. The virus showed no signs of dying out. It gave no evidence of becoming more virulent. The incubation period remained variable, and the percentage of positives did not increase. All inoculations were by no means successful, and there was great individual difference in susceptibility. Only 40% of the animals succumbed. These authors describe two types of symptoms. In one type, designated as the "progressive," there was paralysis of one extremity progressing until death occurred, resembling somewhat the type of disease found in monkeys and man. In the other group, designated as the "fulminating" type, the symptoms were more explosive in character with extreme weakness and prostration, death coming on within a few hours as a result of respiratory failure. The period of incubation was variable. It did not depend on the route of inoculation. The pathologic changes were different from those in monkey and man so far as microscopic examinations were concerned. "They lacked the distinctive features of the pathological picture of poliomyelitis in man and the monkey. Capillary congestion, punctate hemorrhages, degeneration of the motor cells, satellitosis and more or less cellular infiltration of the gray matter of the cord and medulla are found, but perivascular infiltration is absent and the infiltrating cells are not lymphocytic in character." These authors feel, however, that they were dealing with a modified form of poliomyelitis; that the rabbit reacts differently to the virus than do monkeys and man. They raise the question, therefore, whether poliomyelitis may not occur in other animals in some hitherto unrecognized or unnoticed form.

Krause and Meinicke, and Lenz and Huntemüller had previously reported the successful passage of poliomyelitis virus from rabbit to rabbit through successive generations. They found only slight lesions in the brain and spinal cord. These workers obtained better results by intravenous and intraperitoneal inoculations than by the intracerebral route.

Failure to inoculate rabbits successfully has been reported by Römer and Joseph, Landsteiner and Levaditi, Leiner and von Wisner, and by Flexner and Lewis.

Interesting results were obtained by Marks, who was able to pass poliomyelitis virus through 7 generations of rabbits. The effects in rabbits, however, were not definite. Death occurred in some of these animals in from 7 to 11 days, with development of motor weakness and convulsions just preceding death. Monkeys inoculated with brain suspensions of these experimental rabbits developed similar symptoms. The pathologic changes noted in the rabbits were not characteristic, the only findings being hyperemia of the brain. As Marks states: "The disease thus produced in rabbits cannot be recognized as poliomyelitis." He finds further that "not all strains of the virus can be transmitted successfully to even a small fraction of all varieties of domesticated rabbits."

A 5% unfiltered suspension of poliomyelitis virus was used. This had been obtained from the Rockefeller Institute. Inoculations were made intracerebrally, intraperitoneally and intravenously. The intracerebral route was the one most frequently employed. In some animals the intracerebal route was combined with the intravenous or with the intraperitoneal route. Only young rabbits were used; the average age was 6 weeks.

First Passage.—Ten rabbits, 344 to 353, were inoculated intracerebrally with from 0.25 cc. to 2 cc. At the same time monkey X was inoculated intracerebrally with 0.6 cc. of the same virus. Only one rabbit, 348, developed typical symptoms (6th day); the animal was chloroformed on the 13th day. One rabbit was discharged after 10 weeks, and the remaining rabbits died after from 24 hours to 1 month. Monkey X, used as a control, developed typical symptoms of poliomyelitis on the 5th day and died on the 9th day. Examination showed the changes characteristic of poliomyelitis.

Second Passage.—Pooled brain suspension from 2 rabbits (348 and 350) of the first passage was inoculated into 2 rabbits, 358 and 359, both intracerebrally and intravenously. One of the rabbits (348) furnishing this virus, it will be recalled, was the one which had developed typical, progressive, paralytic symptoms, and the other (350) had been found dead after 7 days.

Brain suspension of monkey X was also inoculated in a second series of 4 rabbits, 354 to 357, (2d passage of virus). The doses varied from 1 cc. to 1.5 cc. The intracerebral route alone was used in this group. None of these animals developed any symptoms.

Third Passage.—The pooled brain suspension from rabbits 355 and 356 was inoculated into a series of 4 rabbits (372 to 375). One of the rabbits (374) developed paralytic symptoms on the 14th day. The symptoms were similar to those observed in rabbit 348 of the 1st passage. Suspension from the brain and cord of rabbit 356 was also inoculated in a 2d set of rabbits (376 and 377), both intracerebrally and intraperitoneally in the one, and intracerebrally alone in the other. No symptoms developed in these animals.

Fourth Passage.—A suspension from the brain of rabbit 374 of the 3rd passage of virus was next inoculated in a series of 10 rabbits (378 to 387). The doses from the intracerebral inoculations varied from 0.25 cc. to 0.5 cc., and all rabbits of this series except one were also inoculated intraperitoneally with 2 cc. of the suspension. All of these rabbits died in from 15 minutes to 4 days. No symptom developed.

Fifth Passage.—A pooled brain and cord suspension from rabbits 378, 379, 382, 383, and 387 was inoculated in a series of 4 monkeys (93, 52, 53 and 31) and 6 rabbits (394 to 399). The monkeys were given 1 cc. of suspension intracerebrally and from 3 to 20 cc. intraperitoneally. The rabbits were given 0.4 and 0.5 intracerebrally and 5 cc. intraperitoneally. In none of the animals of this series did any symptoms develop. The virus had apparently died out.

Pathologic Changes.—Tissues of rabbits 350, 351 and 353, examined by Christine I. Gruggel, showed a slight amount of neurophagocytosis of the ganglion cells and some cell degeneration. There was no evidence of round cell perivascular infiltration. As the degeneration of the ganglion cells might be due to other causes, there was no conclusive evidence of poliomyelitis, but it cannot be excluded.

In rabbits 344, 345, 348, 373, 374, 375, 377, 395 the meninges were normal; in the gray matter occasional ganglion cells showed cytolysis and neurophagocytosis, the latter being mononuclear. There was no cellular infiltration or evidence of poliomyelitis.

Sections of monkey X showed a round cell infiltration of the meninges following the vesels into the gray matter of the anterior horns. In places were patches of exudate in which was degeneration of ganglion cells, with neurophagocytosis—a typical picture of poliomyelitis.

Discussion

In only 2 rabbits did symptoms occur that were at all comparable to those in poliomyelitis of monkeys and of man or that corresponded with those described by Rosenau and Havens in their "progressive paralytic" type. One of these rabbits (348) of the 1st passage of virus developed symptoms after an incubation period of 6 days. There was weakness of the posterior extremities, which were held wide apart. There was definite paralysis of the left foreleg and paresis of the right foreleg. This animal was chloroformed on the 13th day. Necropsy did not show any definite lesions, and the pathologic examinations showed only some cytolysis of the ganglion cells and mononuclear neurophagocytosis. There was no cellular infiltration. Cultures to determine the presence of aerobic and anaerobic micro-organisms were all negative. Rabbit 374 of the 3rd passage of virus developed similar symptoms on the 14th day. This rabbit had been inoculated with virus passed through monkey X, used as a control in the first passage; the changes were the same as in rabbit 348.

Of the remaining 26 rabbits which succumbed, 5 died within 24 hours or less. These must be left out of consideration, as death may have been due to injury or shock. In the case of 3 rabbits dying almost immediately, anaphylactic shock due to a foreign protein may have been the cause. In the case of 21 rabbits surviving the first 24 hours and living in some cases as long as a month, it is impossible to determine the cause. Some of them exhibited the symptons described by Rosenau and Havens as of the "fulminating" type. Some of them were found dead without any symptoms having been noted. In the necropsis and examinations of tissue sections of these animals, no changes characteristic of poliomyelitis were found; at least there was an absence of such cnanges as are found in poliomyelitis of monkeys and man. The dose and the route of inoculation does not seem to have caused any difference in the effect. The period of incubation was variable in all passages. There was apparently no change in the virulence of the virus.

The question of anaphylactic shock was carefully considered in carrying out these experiments. A series of 6 rabbits, 338 to 393, were inoculated with 5% suspension of normal rabbit's brain. None of these animals developed any symtoms, and they were discharged after a period of 2 months. This may, however, be merely a coincidence. It may be that none of the animals in this small group of controls were sensitive to the brain protein of the particular group of animals used.

It is difficult to make any definite deductions from the results of the foregoing investigation. If the rabbits exhibiting the "fulminating" type of symptoms died as a result of poliomyelitis virus, we should be forced to accept the suggested hypothesis of Rosenau and Havens that in some species of animals, poliomyelitis develops or assumes a type of disease hitherto unrecognized or unnoticed. Even in the 2 rabbits which developed symptoms similar to those occurring in poliomyelitis of monkeys and of man, the histologic changes were not characteristic. As, however, in both types of effects certain histologic changes were present, it cannot be denied absolutely that they were caused by poliomyelitis virus. The changes present, although different from those found in monkeys and man, may nevertheless be the changes produced by poliomyelitis in rabbits. The fact that one of the rabbits developed typical symptoms from virus that had been passed through a monkey and one generation of rabbits, would certainly suggest such a possibility. The symptoms and pathologic changes were the same as in the case of the rabbit of the first passage inoculated directly with fixed virus.

SUMMARY

In the experiments on young rabbits, symptoms resembling poliomyelitis were observed in a small number. A larger number of rabbits died after varying periods of incubation, but without showing characteristic symptoms of poliomyelitis. The virus did not show any increasing virulence by passage through rabbits. In one series of animals, the virus apparently died out after one passage. In the other series, it apparently died out after passage through a monkey and 3 sets of rabbits. Brain suspension from this 3rd passage through rabbits, inoculated in both monkeys and rabbits, failed to have any effect. In the case of the animals which died, the factor of infection was carefully controlled by cultures; also the possibility of anaphylactic shock due to a foreign protein was taken into account by inoculating a series of young rabbits with normal rabbit brain suspension. All of these controls were negative. It cannot be said that we have been able successfully to inoculate rabbits with poliomyelitis.

For bibliography see original article.

THE RELATIONSHIP BETWEEN DIFFERENT ANTIBODIES OF HEMOLYTIC STREPTOCOCCI FROM SCARLET FEVER.

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ANNA W. WILLIAMS, CAROLINE R. GURLEY, ET AL.

Studies on strains of hemolytic streptococci from different cases of scarlet fever, from normal throats and from cases of other diseases are being carried on by workers in our laboratory along three main lines:

1. Grouping by agglutination, and absorption of agglutinins. In these studies we have been assisted by H. D. Hussey, F. Bittman, F. Berman, A. Brassel and others.

2. Grouping according to the protective power of serum stimulated by the inoculations of whole cultures. This part of these studies has been carried on mainly by C. R. Gurley, M. F. Carmichael and F. Berman.

3. Grouping according to the power to produce toxic filtrates neutralized by convalescent scarlet fever serum, and by serum from horses inoculated with a toxic filtrate. We have been assisted in this work by H. D. Hussey, A. Brassel, B. Kolchin and others. Most of the human inoculations were made by A. Zingher and R. G. Freeman.

The main purpose of all of these studies has been to determine the relationship of hemolytic streptococci to scarlet fever and to so-called natural immunity from scarlet fever.

In regard to grouping by agglutination, we have found that out of about 70 strains tested, only about 30 per cent. fall into one group; 7 per cent. consist of two small groups, one of 3 strains and one of 2 strains; about 63 per cent. do not fall into these groups.

The strain of Dochez, Avery and Lancefield "No. 273" which they first listed in their work on agglutination[1] as coming from a case of scarlet fever belongs, according to the absorption test, to our heterogeneous group, but the strain Dochez gave to Dr. Huntoon, who kindly sent it to us, labelled "No. 1372", falls by the absorption test into our dominant group—which we have called Type I group. Two strains kindly sent us by the Drs. Dick labelled "No. 1" and "No. 2" both fall into this Type I group.

Eight per cent. of strains from sources other than scarlet fever fall into our Type I group—2 in 25. One of these was from a wound and the other from an endocarditis case.

In view of the fact that only about 30 per cent. of our strains from scarlet fever fall into one group, we conclude that the agglutination test, whatever the significance of the occurrence of such an agglutination group among scarlet fever strains, is not of much practical worth in the diagnosis of scarlet fever or in the identification of carriers.

In regard to grouping according to their power to stimulate the production of antibodies protective in mice against whole cultures, all of our strains from scarlet fever so far tested and our 2 strains from sources other than scarlet fever fall into one group.

We have not studied by this test many of the strains studied in our agglutination work, chiefly because of the difficulty in raising the virulence of some strains, but we have tested enough strains to demonstrate that this power to stimulate the production of protective antibodies is probably broader than the power to stimulate agglutinins.

Thus, while our two strains F and K from other sources, one from a wound and one from endocarditis, are controlled by our Type I serum, one of our measles strains M 22—which falls into our heterogeneous agglutination group is controlled as well. We have so far raised the virulence of 19 strains to a ten millionth of a cubic centimeter or less as a lethal dose for mice—13 from scarlet fever and 6 from other sources.

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[1] *Exper. Med.*, 1919, 29, 215.

In regard to the third line of these studies—studies on toxic filtrates—we have tested many strains of hemolytic streptococci for their power to produce neutralizable toxic filtrates.

We have found that not only do all of our scarlet fever strains tested, which were isolated over 3 years ago, produce such a toxic filtrate, but all pyogenes strains isolated from a very recent series of scarlet fever cases do the same.

Our two strains from sources other than scarlet fever that fall into our protective group, and therefore into our agglutination group, do the same. Also 2 strains that are not protected by our Type I serum and that do not fall into our agglutination group—one from osteomyelitis and one from bronchitis produce neutralizable toxic filtrates.

Some strains from sources other than scarlatina, but which belong to the pyogenes group, and a few outside of the pyogenes group have either not produced toxic filtrates or have produced toxic filtrates that are not neutralized by scarlet fever convalescent serum or by our horse serum.

We have examined a series of 25 cases of excised tonsils with a history of no recent exposure to scarlet fever. From 56 per cent. of these (14 cases) hemolytic streptococci were isolated, and 42.8 per cent. of these (6 cases) produce toxic filtrates neutralized by convalescent scarlet fever serum. These strains have not yet been tested by agglutination or by mouse protection.

In this connection it is interesting and significant to report on our comparative results in protection in mice against the living culture with serum from horses inoculated with toxic filtrates, and from those inoculated with whole cultures of these streptococci from scarlet fever. Our whole culture streptococcus serum affords marked protection in mice against all of our strains protected by our Type I rabbit serum, while our toxic filtrate horse serums, which are yielding a serum having a high neutralizing value, do not yet protect at all.

The serum from Dochez' first bleedings that he sent us do not protect either, but Dochez now states[2] that he has a serum that protects against the whole culture and neutralizes the toxic filtrate as well.

These facts add to the evidence in favor of certain hemolytic streptococci being the cause of "scarlet fever."

But they point toward the probability of "scarlet fever" being only one of the manifestations of infection by a large group of these cocci.

They suggest that, while immunizing with toxic filtrates may prevent clinical scarlet fever, it may not prevent the more serious effects of hemolytic streptococcus infections. They also suggest that serum stimulated by the inoculations of toxic filtrates alone may not be so effective a curative agent as one stimulated by the simultaneous inoculations of toxic filtrates and whole cultures of the streptococci.

They finally raise the interesting question as to the possibility of there being strains of hemolytic streptococci in the throats of many people which, while not causing clinical scarlet fever, may produce immunity from such a condition.

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[2] Personal communication.

SCARLET FEVER

Etiology, Prevention by Immunization, and Antitoxic Treatment*

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William H. Park, et al.

The etiology of scarlet fever has been under active investigation for many years. Loeffler, in 1884, and later other bacteriologists noted that the hemolytic streptococci were very abundant in the throats of acutely ill scarlet fever patients. Clinicians also noted the fact that in certain cases of wound infection and puerperal fever a scarlet fever-like rash developed. These observations led to the opinion that there was a close relationship between certain strains of hemolytic streptococci and scarlet fever. The majority, however, held that the hemolytic streptococci were merely very important secondary invaders. Repeated attempts by numerous investigators to produce any disease similar to scarlet fever in animals by the inoculation of throat secretions of cultures of hemolytic streptococci obtained from the throats of scarlet fever patients failed. It was therefore necessary to turn to tests on man.

Moser reasoned that, if the streptococcus was the cause of scarlet fever, a curative serum might be produced, and in 1902 published a report of the good therapeutic results obtained by the use of an antiscarlet fever serum produced by the horse after frequently repeated injections had been made of living streptococci together with the broth in which they had developed, so as to get the antigenic value of the organisms as well as of any toxins that might have developed in the broth. The strains of streptococcus used were obtained from a number of toxic cases of scarlet fever.

A Russian investigator, Savchenko, went a step further; he showed, in 1905, that the serum contained both scarlet fever antitoxin and streptococcic bactericidal bodies. He also proved that the filtered broth in which the culture had grown contained a strong toxin. He said:

"Immunizing with the microbes only, it is possible to obtain a very active antimicrobal serum, but not at all antitoxic; vice versa, by immunizing with a toxin, we obtain an antitoxic serum and, therefore, one not acting on the microbes.

In scarlet fever, in which we have severe local infections and at times even general infections together with the toxemia, we must have in the serum antitoxins as well as germicidal antibodies. Filtrates of our own cultures of streptococci from scarlet fever grown in different combinations of broth proved to be nonpathogenic for rabbits, even in large doses. To conclude from this that there is no poison in the medium would be just as erroneous as to infer that there is no tetanus toxin after we had tried it on chickens, which are insusceptible to it.

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* This study of scarlet fever was aided by a grant of money from the Milbank Memorial Foundation.

And yet Moser, using broth cultures, obtained a serum which unquestionably acted on human beings antitoxically. It is obvious that either the streptococci produced a toxin in the medium used by Moser, or the toxin developed in the animal's system after its infection with living bacteria. In the latter case the method of immunization would have to be changed in the direction of the greatest possible increase of the number of bacteria in the material to be injected.

. Therefore, before definitely choosing the kind of medium and the method of immunization, we made a few experiments with filtrates on the same horses that were subsequently used for immunization.

Martin's broth, which is being used for diphtheria toxin, was used as a culture medium in this experiment. The growth may be improved by adding to the broth either 0.5 per cent. dextrose or 2 per cent. ascitic fluid. Though the addition of sugar increases the growth of the streptococci considerably more than does the addition of ascitic fluid, it (by the products of fermentation) changes the reaction of the culture, making it acid. It should be expected, as is the case with diphtheria toxin, that this will prevent the formation of toxin. Indeed, an experiment on horses shows that filtrates of cultures with sugar are very little toxic, whereas filtrates of broth cultures with ascitic fluid clearly showed toxicity:

Two horses were injected with 15 cc. of filtrates of four days' broth cultures in the following order: Horse 1, fifteen cc. filtrate of broth sugar culture containing an abundant number of streptococci; Horse 2, fifteen cc. filtrate of broth ascitic culture containing a moderate number of streptococci. After twenty-four hours, only a faintly noticeable swelling was seen at the site of inoculation of Horse 1, and no rise of temperature was observed. Horse 2 developed quite a considerable edema and a rise of temperature. When the reaction from inoculation in Horse 2 disappeared, the injection of the same amounts of the same filtrates were repeated, but in a reversed order. Horse 1 developed a very strong reaction, whereas Horse 2 did not react at all.

It is obvious that cultures grown on 2 per cent. ascitic broth are preferable for toxin production to those grown on sugar broth, even if the organisms are less luxuriant.

The same filtrate, injected under the skin of rabbits in amounts of 5 and even 10 cc., produced no symptoms except a slight swelling. The filtrate turned out to be just as harmless for other laboratory animals.

These experiments showed that it is possible to obtain a sufficiently strong toxin, but they unfortunately also showed that by means of our laboratory animals it is impossible to gage either the amount of toxin—which is so important for proper immunization—or the amount of antitoxic substances in the serum, which is not less important for curative purposes. For the time being we are compelled to work, so to speak, by "feeling" our way.

As long as the broth cultures were shown to contain toxin, it appeared most proper to immunize horses with living cultures with the accompanying toxic broth, because in this way both antitoxic and antimicrobal substances should be obtained."

After reading the paper of Savchenko, no one can doubt that he produced by the filtrate a true antitoxic serum.

HUMAN IMMUNIZATION AGAINST SCARLET FEVER AND VALUE OF THIS EVIDENCE IN FAVOR OF THE CAUSAL RELATIONSHIP OF STRAINS OF STREPTOCOCCI TO SCARLET FEVER.

Gabritschewsky had finished an experimental study on a streptococcic infection of horses in which an appreciable rash developed, and believed he had proved that a vaccine of toxin and dead streptococci was able to develop a moderate degree of immunity. He then turned to the question of utilizing a streptococcus vaccine made from the toxin and cells of streptococci obtained from scarlet fever. His vaccine was made of bouillon, in which the streptococci had grown for four days, plus the streptococci themselves. The toxic bouillon containing 3 per cent. by volume of the streptococci was heated to 60 C. to kill the streptococci, and then preserved with 0.5 per cent. phenol (carbolic acid). Three doses were given subcutaneously one week apart. For infants under 2 years the dose was 0.25 cc.; for children between 2 and 10 years, 0.5 cc., and for those over 10 years, 1 cc. The results in children were most important. Langowoi, using his vaccine, found that of 120 childen inoculated, 13.3 per cent. developed a fine scarlet fever-like exanthem which was not followed by desquamation. A few developed a sore throat and strawberry tongue, and fewer still vomited. He states that these symptoms are those which are characteristic of scarlet fever, and these results of the vaccination add an extremely important proof "for the specificity of a scarlet fever streptococcus and its toxin." There is no question that Gabritschewsky considered the bouillon to contain a true toxin, not only from his language, but also because he was in contact with Savchenko, and their articles appear together a little later in the same Russian journal. He found that the second and third injections, although they were twice and four times as large as the first, rarely produced a rash. This he considered as evidence of the rapid development of immunity.

Gabritschewsky, in 1907, gives further evidence in favor of vaccination. Of 248 children receiving injections of the vaccine, Nikitin noted the rash in 17.3 per cent. In six he noted a generalized rash, vomiting, a strawberry tongue and an angina. In seven cases, the lymph glands were slightly swollen; in ten cases, albumin appeared in the urine for a day. In one case there was an acute nephritis, which ended in recovery. Twenty-six children who had previously had scarlet fever showed either no reaction or only a slight local swelling. A group of children tested during their fourth week of convalescence showed a greater percentage of reactions than those who had been convalescent for a long time. At Gabritschewsky's suggestion, Langowoi vaccinated twenty children with a vaccine made in the same way as the scarlet fever vaccine from the streptococcus of erysipelas. None of these developed a rash. These case are too few to prove that the result would have been the same if a larger number had been vaccinated, but they are suggestive of the specific character of the scarlet fever toxin. Gabritschewsky closes his article with five conclusions, of which I quote the last two:

"The fact that a scarlet streptococcus vaccine (toxin and culture) is able to produce a scarlatiniform rash and the other symptoms of scarlet fever is a decisive proof in favor of accepting a streptococcus as the specific cause of scarlet fever.

This decisive proof adds a scientific reason for the use of Moser's serum and my vaccine."

Gabritschewsky's death prevented him from following up the practical results of his vaccine. The following statement by Polotevkova is one of several encouraging reports:

"A severe outbreak of scarlet fever took place in my district, with 1,200 cases of the disease. Not only children, but also adults between 20 and 50 years of age were among the sick. The form of the disease was also severe, very often involving suppurative affections of the mouth, nose and upper respiratory tract. Owing to the extreme poverty of the peasants of this district, where very often milk could not be obtained for the sick, the only hope was in the preventive immunization. In short, the results were such that in the villages in which vaccination took place only one or two or no cases at all were observed; in villages in which no vaccination was carried on, the number of sick would reach from six to 175 cases. The author vaccinated her own three children."

Gabritschewsky considered that he had added to the proof already gathered sufficient evidence so that scarlet fever could be considered to be in the same class as diphtheria. The scarlet fever streptococcus produced in toxins in the throat. The absorbed toxins produced the rash, the fever and the other symptoms due to toxemia. The lowered resistance allowed the scarlet fever streptococci or other streptococci to invade distant parts and make the so-called complications. Additional evidence of the casual relationship was the development of a case of scarlet fever by the accidental inoculation in our laboratory of the throat of one of the assistants by a culture of several strains of streptococci including a scarlet fever strain. A typical case of scarlet fever developed, but attempts to reproduce the disease in animals with the culture failed.

In 1918, Schultz and Charlton described the reaction known by their name. When 1 cc. of a diluted convalescent serum is injected intracutaneously into the reddened skin of a scarlet fever patient, a blanching occurs in an area several centimeters in diameter. In December, 1923, Mair published further results and concluded that this reaction is due to the action of an antitoxin on the scarlet fever specific toxin. This brings us to the discoveries of the Dicks and of Dochez. In spite of the proof, which was largely unknown to most workers, the majority still believed that the hemolytic streptococcus found in scarlet fever was only an important complicating infection. In October, 1923, the Dicks inoculated five volunteers, who had lived in the country, with a streptococcus culture from a case of scarlet fever. One developed a typical case of moderate scarlet fever. The throats of five others were inoculated with the filtrate of a broth culture. None of these developed the disease, but when the same persons were inoculated with the streptococcus, one developed it. This proved that the infection agent was not a filtrable virus attached to the streptococci. It was found by them that the streptococci isolated by them from scarlet fever differed from one another in the ability to ferment mannite. The first two cases had been caused by an organism that fermented mannite. The Dicks then tried out the effect in the human skin of the toxin discovered by Gabritschewsky and Savchencko. They found that weak solutions of the toxin may be employed in skin tests to determine susceptibility to scarlet fever. The toxin is first carefully standardized and diluted so that 0.1 cc. represents a skin test dose. The test consists of an intradermal injection of exactly 0.1 cc. of the skin test dilution on the flexor surface of the forearm. The reaction is observed at the end of twenty-four hours. An area of reddening 2 cm. in diameter indicates marked susceptibility; 1 cm. in diameter, some degree of susceptibility to scarlet fever.

In a series of skin tests, reported in January, 1923, the Dicks found a positive or strongly positive reaction in 41.6 per cent. of the persons who gave no history of scarlet fever, and negative or only slightly positive reactions in all the convalescent fever patients tested. Zingher's observations fully corroborate these findings and show that the results in age groups from the Dick test were very similar to the results from the Schick test.

This skin test was developed before they attempted to produce experimental scarlet fever with the type of streptococcus that does not ferment mannite. In selecting their volunteers for inoculation with this type of streptococcus, they chose one with a negative skin reaction and one with a positive reaction. The two volunteers were inoculated with the same culture. The one with a negative skin test remained well, while the volunteer who had shown a positive test developed scarlet fever.

In the instances in which they had an opportunity to observe the skin reaction before and after an attack of scarlet fever, it was positive before the attack, and negative during convalescence. It is now known that there are about 10 per cent. of exceptions to that rule.

These authors also found that if persons with positive skin tests received convalescent scarlet fever serum intramuscularly, their skin tests became negative. With more definite doses than Gabritschewsky used they then attempted to immunize persons against scarlet fever and utilize later their skin test to determine the development of antitoxic immunity. They found that, by proper dosage, they were able to immunize persons with positive skin tests so that their skin tests became negative and they did not contract scarlet fever on exposure.

They report a series of 125 persons exposed to scarlet fever. In this series, sixty-three showed negative skin reactions and were not immunized. Fifty-two had positive skin tests, and were immunized with toxin. None of these persons contracted scarlet fever. They emphasize the importance of carrying the immunization to the point of a negative skin test. By the employment of three graded doses of toxin, they have been able to eliminate severe reactions.

While the Dicks were adding to the proof as to the causal relationship of certain strains of streptococci to scarlet fever, Dochez was developing an antitoxic serum through a method, developed by himself, of injecting subcutaneously into animals, masses of nutrient agar infected with scarlet fever streptococci. These injections, repeated from time to time in increasing amounts during from six to eight months, were found to develop a potent antitoxin. The serum of horse that had been subjected to such injections was found to give the Schultz-Charlton test, even when the serum had been diluted several thousand times. The serum was used in cases in New Haven and in New York, and the same results were regularly obtained as had been formerly obtained with unstandardized serum by Moser and Savchenko; but now the potency of the serum was established by the striking results with the Schultz-Charlton reaction. These were a demonstration to all that antitoxin was truly present. The interest developed by Dochez's announcement caused a number of biologic plants to use his methods, while others utilized toxin standardized by the Dick test, or crude toxic broth and dead organisms after the manner of Moser and Savchenko.

Shortly after Dochez announced his serum, the Dicks made a report on the production of antitoxin by the toxic filtrate standardized by their method. Whether the Dochez method or the various modifications of the toxin with or without killed or living streptococci will finally be adopted remains for further comparative tests to decide. This will depend largely on the value of an antimicrobal substance in the serum. The results with the Dochez serum or the Dick serum agree absolutely with the reports of the use of the Moser serum. The Dicks have suggested that the antitoxin be standardized by the number of skin doses it neutralizes. The federal government has accepted this, and a tentative unit has been adopted by antitoxin producers, but not yet accepted by the Dicks. A unit is defined as the amount of antitoxin that is required to neutralize 50 skin test doses to the extent of preventing a reaction of one cm. in diameter of scarlet fever toxin. This is determined by the method suggested by the Dicks of mixing 2 cc. of a 1:10 dilution of serum with 2 cc. of a dilution of standard toxin (200 skin doses) and injecting 0.1 cc. At the same time a control Dick test and a control serum test are made, to rule out protein and serum reactions. If the patient gives a good Dick test, no serum reaction and no other non-specific protein reaction, 1 cc. will neutralize at least 2,000 skin doses and contains at least 20 units. If neutralization occurs, higher dilutions of serum are used for further tests.

ACTIVE IMMUNIZATION

Earlier in this paper the Author gave the work of Gabritschewsky and his followers. In the light of the Dicks' researches and the rashes that developed, it can be estimated that the Russian infants received about 2,000; 4,000 and 8,000 skin test doses and older children 4,000; 8,000 and 16,000, or

TABLE 1.

IMMUNIZING RESULTS OF THREE INJECTIONS OF 300, 500 and 1,000 SKIN TEST DOSES OF SCARLET FEVER TOXIN GIVEN AT INTERVALS OF ONE WEEK

	Results of Retest	
Before Injection Reaction to Dick Test	Two Weeks After Last Injection	Four Weeks After First Injection
	2=2+	
	2=+^{1}	50 per cent. immune
10 ++ or ++^{1}	1=+	
(Strong reactors)	5=−	50 per cent. still susceptible
	4=+	68.5 per cent. immune
35 +^{1} or +	7=±	20 per cent. doubtful
(Moderate reactors)	24=−	11.5 per cent. probably susceptible

RESULTS WITH INJECTIONS OF DOUBLE THE FOREGOING AMOUNTS

Before Injection	Two Weeks After First Injection	Four Weeks After First and Two Weeks After Third Injections
12 ++ or ++^{1}	3=2+	2=+^{1}
(Strong reactors)	3=+Week	1=±
	2=±	9=−
	3=−	
	1 not tested	
	27 per cent. immune	75 per cent. immune, 16 per cent. not immune, 8 per cent. doubtful

++ Indicates a Dick reaction of 2 cm. in diameter and + of 1 cm.
+^{1} Indicates more than + and ± less than +.

perhaps double that amount. With the Dick test, the dosage can be standardized and the immunity results determined by retesting from time to time. The scarlet fever toxin is much less injurious to the local tissues than the diphtheria toxin, so that there is no need of adding antitoxin to it or of changing it by formaldehyd. Yet, as the Dicks state, such chemical modification, resulting in a decreased toxicity or toxoid, is desirable, if it can be demonstrated that it accomplishes immunization with fewer doses or in a shorter time. The most suitable dose of toxin varies with conditions. If scarlet fever is prevalent, it is very desirable not to give sufficient toxin to make a rash in any of the very susceptible children, for there is no certain way of determining in such a case whether it is a case of light scarlet fever or of a toxin rash. Even if cultures are made, it is possible that streptococci which produce the scarlet fever toxin will be present, and yet the case will be due to a toxic rash. If, however, no scarlet fever is in the neighborhood, a slight scarlet rash is of no importance and larger doses can be given.

TABLE 2.

RESULTS IN DICK POSITIVE CHILDREN THREE AND ONE-HALF MONTHS AFTER RECEIVING THREE IMMUNIZING INJECTIONS OF APPROXIMATELY 1,000, 2,000 AND 4,000 SKIN DOSES

	Reaction Three and One-Half Months Later	
11 + + or + +[1] (Strongly positive)	1 = +	63 per cent.. immune
	3 = ±	27 per cent. doubtful
	7 = —	9 per cent. probably suspectible
10 + or +[1] (Moderate reactors)	1 = ±	90 per cent. immune
	*1 = pseudo	10 per cent. doubtful
	8 = —	
Total 21 cases	1 = +	76 per cent. immune
	4 = ±	19 per cent. doubtful
(Strong and moderate reactors)	15 = —	4 per cent. moderately susceptible
	1 ± pseudo negative	

* Gave + reaction with and without mixture with a sufficient quantity of convalescent serum to neutralize the toxin.

TABLE 3.

RESULTS OF FIVE IMMUNIZING INJECTIONS AT THE END OF THREE AND ONE-HALF MONTHS APPROXIMATELY 500, 1,000, 2,000, 3,000 AND 4,000 SKIN TEST DOSES

	After Three and One-Half Months	
32 + + or + +[1] Strongly positive cases	1 = +	81 per cent. immune
	5 = ±	= 17 per cent. probably immune
	23 = —	= 2 per cent. probably susceptible
	*3 + ± negative pseudo	
36 + or +[1] Moderate reactors	34 = —	
	*1 = + negative pseudo	100 per cent. immune
	*2 = ± negative pseudo	
69 + to + +[1] All positive reactors	1 = +	91 per cent. immune
	5 = ±	7 per cent. probably immune
	57 = —	1 per cent. probably susceptible
	1 = + negative pseudo	
	5 = ± negative pseudo	

* 1 + and *5 ± are considered negative pseudo reactions because of an equal reaction in the control neutralized by convalescent serum.

Original Investigations.—Tables 1, 2, 3, 4 and 5 show the results in our tests following the injection of from 600 to 10,500 skin doses. The tests in these tables, with the exception of the one by Zingher, were made by Schroder and read by her, Williams and the Author.

The persistence of the acquired immunity following injections remain in doubt. With small injections, such as in the cases recorded in Table 5, many of the originally very positive cases have, after a year, relapsed, while the originally moderately positive cases have generally continued to hold their immunity or to increase it.

TABLE 4.

IMMUNITY RESULTS AFTER FOUR WEEKS WITH THREE INJECTIONS OF SCARLET FEVER TOXIN (DICK TEST)—1,200 SKIN TEST DOSES IN ALL (ZINGHER)*

Dick Retest After from Four to Five Weeks

			Dick Positive and Combined			Dick Negative and Pseudo	
Institution	Per Cent. Positive Dick at Original Test	Total Retested	Number Positive As in Original Test	Number Less Strongly Positive	Per Cent. Less Strongly Positive	Number	Per Cent.
Hebrew Orphan Asylum..	29.2	143	19	20	14.0	104	72.7
New York Orphanage....	44.4	91	10	36	39.5	45	49.4
Leak and Watts Home....	22.0	40	12	10	25.0	18	45.0
Total		274	41	66	24.0	167	61.0

* Doses given: under 12 years, 200, 500 and 500 for skin test doses at weekly intervals; over 12 years, 200, 500 and 1,000 skin test doses at weekly intervals.

TABLE 5.

RESULTS OF INJECTING 150, 300 AND 600 SKIN TEST DOSES AS SHOWN BY RETEST FIVE AND THIRTEEN MONTHS AFTERWARDS AT ST. DOMINICKS

	Five Months After	Per Ct.	Thirteen Months After	Per Ct.
One hundred positive cases of St. Dominicks Test by Dr. Schroder	+ + = 0		+ + = 20	
	+ = 8 =	9.8	+ = 9	susceptible
	+ = 27 =	33.3	± = 14	doubtful
	± and − = 46 =	56.8	± or − = 57	immune
	Total 81		Total 100	

With much larger injections, such as those recorded in Tables 2 and 3, the immunity response is so much greater that it is hoped that the marked positive cases will not only develop immunity but hold it for a period of years.

We note in these tables that the moderately reacting cases develop complete immunity much more readily than the strongly reacting cases; again, that the response is quicker than after diphtheria toxin-antitoxin or toxoid injections. This corroborates the views of Gabritschewsky and the statements of the Dicks that immunity develops in many within one or two weeks.

The results of a second series of injections in those who have not responded to the first series have been excellent.

The persistence of a negative Dick reaction in those found to be negative: Observations on several thousand children by Zingher and Schroder at different intervals of time up to thirteen months have shown that a naturally acquired immunity is usually persistent for at least one year. Only about 2 per cent. of those originally negative have been found to change. It is fair to assume, therefore, that as in the case of naturally acquired immunity to diphtheria, the immunity to scarlet fever naturally acquired is apt to remain. This finding, which is in accord with the findings of the Dicks, encourages us to hope that, with larger and perhaps more numerous doses, even the very positive cases may acquire such a high degree of immunity that it will last for at least a few years. We already have the knowledge that these immunizing injections are very useful in stopping an outbreak of scarlet fever.

The Serum Treatment of Scarlet Fever as Observed at the Willard Parker Hospital

Scarlet fever antitoxin was first administered by us in January, 1924. This was made possible through a supply of serum by Dr. Dochez. The first serum was only moderately potent and, in the doses given by the intramuscular method, the results though good were not striking. In January, 1925, it was decided to give it to alternate patients, and in severe cases to give it intravenously. The antitoxin most used was our own preparation obtained from two horses that had received increasing toxin injections for nine months and more; but in about one-fourth of the cases we used a new preparation of the serum sent us by Dochez. The result of the intravenous injection was so striking that the medical board decided in March, 1925, to give antitoxin to all patients having a rash and a temperature of 101 F. or higher and to give it to alternate patients having a temperature lower than 101. It was given intravenously in all severe cases.

The antitoxin serum was given by Dr. Mildred McBride, the assistant resident physician who was in charge of the scarlet fever service at the Willard Parker Hospital. The attending physician on duty and the Author watched with her the effects of the treatment. The potency of the antitoxic serum was tested either by the Schultz-Charlton blanching test or by the more accurate Dick neutralization tests of the toxin. The potency ranged from 100 to 300 units per cubic centimeter; that is, 1 cc. of the serum neutralized between 5,000 and 15,000 Dick test skin doses of toxin.

Most of the serum was not refined. About fifty patients, however, received the refined antitoxic globulin solution. The therapeutic results were alike, but rashes occurred in about 60 per cent. after the injection of the unrefined serum, and in only about 30 per cent. after injection of the refined. The rashes and other symptoms of serum disease that did occur were more severe with the unrefined. No serious after-effects occurred in any case, but the serum sickness in some was very annoying. The dosage was determined by much the same principles that govern the dosage of diphtheria antitoxin. A sufficient dose should be given at the earliest possible moment. An amount should be given in the first dose to make and keep the fluids of the body antitoxic. If, however, the temperature rises after the drop, a second injection should be given. The size of the dose is influenced by the weight of the individual and the severity of the case. Whether a late case in which there is still a marked rash should be treated with larger doses than an early case of equal severity is in our belief doubtful and remains still a matter for further investigation. Intravenous injections of sufficient size give the most striking results. The fluids of the body become quickly antitoxic, as shown by the Dick test. This, when done even an hour or two afterward, is always negative, if a sufficient dose has been given, and remains negative if done later in the disease.

The results from sufficiently large intramuscular injections are certain, but they develop more slowly. The Dick test is positive for from six to twelve hours after an intramuscular injection. It then becomes negative.

The results as noted at the bedside in the majority of patients are very striking. The higher the temperature and the more toxic the case, the more striking will be the results, if the serum is given very early in the disease. After an intravenous injection in an early uncomplicated case, the patient, as a rule, finds within a few hours that the throat is less sore, the mind clears, vomiting ceases, the appetite returns, and the temperature and pulse begin to fall. Within from six to eight hours, a delirious and a very sick patient is often convalescent. With a larger dose given intramuscularly the same results follow, but more slowly. With insufficient dosage there is a less rapid improvement, and the toxic symptoms may return. Our results were practically identical with those reported by Blake and Birkhaug.

The following two cases showed favorable results after intramuscular injections in the first and intravenous injection in the second:

Case 1.—G. C., a boy aged 7, was sick two days with headache and vomiting. A rash developed on the morning of the 23rd. On admission in the afternoon, the temperature was 103 F.; pulse rate, 150; rash marked, and throat inflamed. The Dick test made on admission was positive. The severity was from moderate to severe. The patient was given intramuscularly 40 cc. (about 3,200 units) of Dochez' antiscarlatinal serum. On the 24th the rash was greatly lessened; the Dick test made in the morning was negative, and the temperature was 101 F. in the morning and 100 F. in the afternoon. On the 25th the rash was gone; the temperature, 99.6 F. The patient made an eventful recovery (Chart 1).

Case 2.—A. K., a man aged 32, who had a sore throat on the 17th, with a rash appearing on the 19th, was admitted early in the afternoon on the 20th. The temperature was 102 F. and rose to 104 F.; the pulse rate was 130. The throat was inflamed. Adenitis was present, and the patient was fairly toxic. He received, on admission, 2,400 units of antitoxic serum intravenously. Within two hours he felt improvement. At the end of six hours the temperature had fallen to 98.8 F., the rash had faded and the patient felt almost well. Convalescence was uncomplicated (Chart 1).

Chart 1 is characteristic of uncomplicated early cases of scarlet fever that respond to the serum treatment. In many there is no subsequent rise of temperature. In others, because of complications or serum sickness, the convalescence is interrupted.

Chart 2 gives the temperatures of five patients who received intramuscular injections. It will be noted, as in Case 1 the previous chart, that the drop in temperature is comparatively slow. Two of these patients received insufficient amounts of antitoxin.

Chart 3 gives the temperatures in five early cases of scarlet fever in which intravenous injections were given and the patients reacted favorably. The temperatures of an untreated case are given also for comparison. The rapid drop of the temperature in four of the patients is striking. In the fifth case only 1,500 units was given. The rash remained bright, and the temperature rose again.

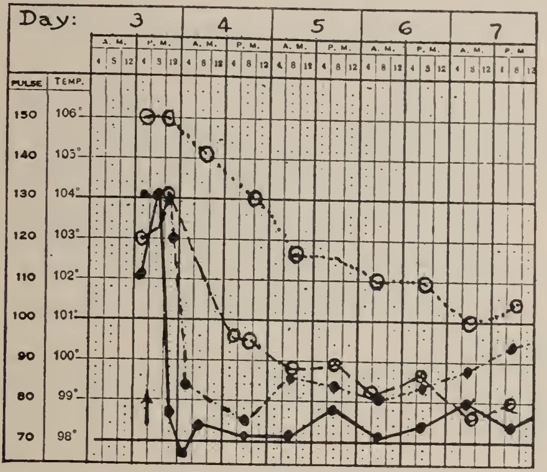

Chart 1.—Pulse and temperature changes in Cases 1 and 2; solid line with solid dots, temperature curve, intravenous injection; broken line, hollow dots, temperature curve, intramuscular injection; broken line, solid dots, pulse curve, intravenous injection; dotted line, hollow dots, pulse curve, intramuscular injection; arrow indicates serum injection.

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Chart 4 gives the composite temperature curves of twenty-five severe, twenty-five moderate and twenty-five light cases of scarlet fever treated with serum during the first three days of the disease and an equal number without.

Complications.—The antitoxin has little effect on complications, such as infection in the tonsils, in the middle ear, in the mastoid, in glands of the

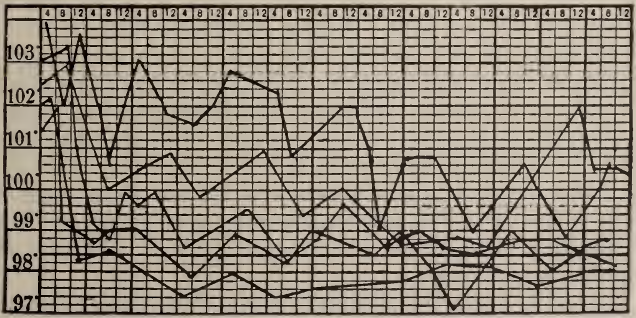

Chart 2.—Temperature curves in five uncomplicated cases after an intramuscular injection of antitoxin. (Reprinted by courtesy of The Journal of The American Medical Association.)

neck or in the endocardium, when they have already developed; but before they have developed the antitoxin appears to be of service in preventing their occurrence. This is probably due to the raising of the resistance of the local tissues and of the body as a whole so as frequently to prevent or restrict the invasion of the body by the streptococci. These streptococci may be those producing the scarlet fever or other varieties. An interesting case was that of a woman sick with toxic scarlet fever complicated by severe erysipelas. The delirium vanished, the vomiting ceased, the temperature was lowered and the rash faded, but the erysipelas ran its usual course. Table 6, which shows the complications developing in seventy-five cases that were treated and in seventy-five that were untreated, indicates a favorable influence of the antitoxin in preventing complications. As long as the rash remains, antitoxin is indicated.

TABLE 6.

COMPLICATIONS DEVELOPING IN SCARLET FEVER IN SEVENTY-FIVE CASES UNTREATED WITH SERUM AND IN SEVENTY-FIVE EARLY CASES TREATED WITH SERUM

	Nephritis	Otitis	Adentis	Arthritis	Mastoiditis
Twenty-Five Severe Cases:					
Without serum	9	12	8	9	5
With serum	2	6	11	5	3
Twenty-Five Moderate Cases:					
Without serum	9	8	8	8	0
With serum	0	7	11	4	0
Twenty-Five Mild Cases:					
Without serum	4	0	8	3	0
With serum	0	4	6	2	0

The cases in Table 6 are not strictly comparable, since the severe and moderate cases which were not treated with serum are taken from 1924, while the serum-treated cases occurred in 1925. The diagnosis of nephritis is based mostly on the urine examinations and is probably not wholly accurate. The kidney complications, on the whole, are seen to be decidedly less in the cases treated with antitoxin early, but the other complications are about the same.

Reduction in Mortality.—During the year 1924, when scarlet fever was very light, 569 patients with scarlet fever were admitted to the hospital. About forty of these received scarlet fever antitoxin. The mortality among the 569 patients was 2.8 per cent. During April and May of this year, when all patients with cases of any severity and with a rash were receiving serum, there were 220 admissions. Of these, six patients, or a percentage of 2.7, died.

These figures, considered without a knowledge of the cases, would be very misleading; but they cause one to wonder what proportion of the 150 or more deaths from scarlet fever which occur yearly in New York can be eliminated by the use of serum. In three of the six fatal cases the patients were admitted so late that they were absolutely unsuitable for antitoxin, but in three of them they were admitted on the third day. The histories of these six fatal cases are as follows:

Case 3.—A child aged 11, admitted thirty-eight days after the development of scarlet fever, showed symptoms of meningitis. Death occurred three weeks later. Necropsy showed several small brain abscesses.

Case 4.—A child, aged 7, admitted on the ninth day of disease, with double otitis media, septicemia and signs of endocarditis, died in three days.

Case 5.—A man, aged 35, admitted in the afternoon on the sixth day of the disease, with an intense rash still present, and a temperature of 104.8 F., received 60 c.c. of Dochez serum intravenously (about 5,000 units). The temperature dropped within six hours to 99.5 but rose the following afternoon when he died. Necropsy showed purulent bronchitis and toxic changes.

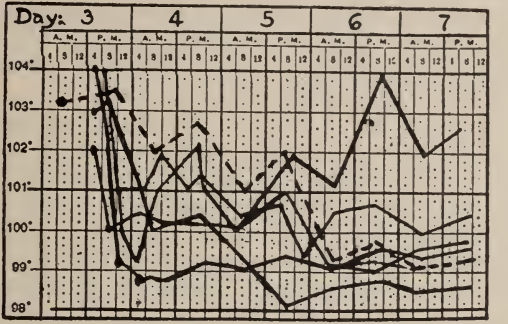

Chart 3.—Temperature curves in five early cases after an intravenous injection of antitoxin, and in one untreated case. The continuous lines give the temperature curves of the patients treated by intravenous injections of antitoxin; one patient received too small an injection (1,500 units); the broken line is the temperature curve of an untreated patient.

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Case 6.—A patient, aged 23, ill three days, with a marked rash, marked pharyngitis with grayish exudate and a temperature of 103.6 F., received 30 c.c. of serum intravenously (about 1,800 units). Complete anuria was present during the first thirty-six hours. The temperature dropped to 99 in eight hours following the administration of the serum, but rose again. The rash remained pronounced for three days. The patient died on the fourth day after admission.

Case 7.—A patient, aged 21, following a kidney operation at another hospital, developed scarlet fever, and was sick three days before admission. The patient appeared toxic with a generalized hemorrhagic rash. The temperature was 103 F. The patient was given 30 c.c. of serum (about 2,000 units) intravenously. The temperature dropped 4 degrees in eight hours, but rose

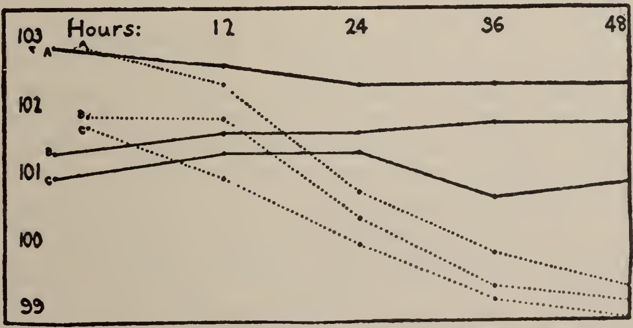

Chart 4.—Average temperatures of seventy-five patients treated with serum and seventy-five patients treated without serum; solid line, patients treated without serum; dotted line, patients treated with serum. There were twenty-five cases of each: *A*, severe; *B*, moderate; *C*, mild.

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in the afternoon just before death. The rash remained, but was somewhat faded.

Case 8.—J. M., aged 3½ years, was taken ill with vomiting, May 10, developed a rash on the 12th, and was admitted on the 13th, with a temperature of 104.8 F., a generalized hemorrhagic rash and an intense pharyngitis with exudate on the tonsils. Several hours after admission, 30 c.c. of antitoxic serum (about 2,400 units) was given intravenously. The temperature dropped to 100 F., but rose to 102 in afternoon. Another 40 c.c. of serum was given intravenously. The temperature dropped to 101, but rose in the evening to 103 and continued between 101 and 103 until death, May 20. The rash did not fade until the 19th. Necropsy revealed an ulcerative inflammation of the mouth, esophagus and stomach.

In the last three of the six fatal cases, the serum was given about as quickly as would generally be possible. Nevertheless, the patients responded only temporarily and inadequately to the antitoxic serum. It is possible that if the serum had been given immediately after the development of the rash, a better result might have been obtained or perhaps much larger or repeated doses of antitoxin might have done more good. There is also the possibility that some of the cases presenting septic rashes are not ordinary scarlet fever and are due to streptococci that differ in the type of their toxin from those produced by the ordinary strains. We have evidence of such difference, but unfortunately we did not isolate and test the streptococci from these cases for their toxins.

Conclusions on the Therapeutic Value of Antitoxin

The antiscarlatinal serum produced by Moser and Savchenko produced the same therapeutic results as that now produced by the Dochez method or by the subcutaneous injection of known doses of toxin. The value of the earlier products was greatly handicapped by the lack of any means such as the Schultz-Charlton and Dick methods for estimating their antitoxic potency. Because of this lack of knowledge, serum weak in antitoxin was undoubtedly frequently used with disappointing results.

The antitoxic serum should be given in sufficient amount as early in the disease as possible. When thus given, the results in most cases are strikingly favorable. The early use of antitoxin probably frequently prevents the development of complications.

Antitoxin is useless after the rash has disappeared and has no effect on the later septic complications.

In moderate cases it should be given intramuscularly. In severe or toxic cases it should be given intravenously. As a rule, a single dose of sufficient size is enough, but in severe cases in which the symptoms return, a second dose in from twelve to twenty-four hours is often desirable. It is too early to state whether the serum should be given in very mild cases, since the serum sickness is often more annoying than the scarlet fever.

The refined antitoxic serum produces fewer and less severe rashes than the unrefined, and should in time be the only preparation used. A unit should be adopted, so that results from different dosages may be compared. The one suggested at a recent meeting of immunologists in Washington seems to be suitable. One unit is defined as the amount of antitoxin that neutralizes 50 skin test doses (Dick), as determined by an intracutaneous test in man. A dosage between 2,000 and 10,000 units will probably suffice in the great majority of cases. A few of the failures of antitoxin may be due to the fact that some toxic cases may be due to streptococci that produce other toxins than are neutralized by the antitoxin used. This is an interesting problem for further study. Whether it will be of marked advantage to combine an antimicrobial serum with the antitoxic serum is still doubtful, but merits careful investigation.

Such a serum used alone in the past has given doubtful results, but combined with antitoxin it may add to the benefit produced. There would thus be a return to a serum such as was used by Moser, but with knowledge of the potency in both the bactericidal and the antitoxic properties.

For bibliography see the original article.

THE DICK TEST IN NORMAL PERSONS AND IN ACUTE AND CONVALESCENT CASES OF SCARLET FEVER

Immunity Results With Scarlet Fever Toxin

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Abraham Zingher

Results with the Dick Test in Normal Persons

The test has been applied to the following groups of individuals, and their reactions have been studied according to age and social conditions:

1. Mothers and young infants.
2. Children of nursery and preschool age.
3. Elementary and high school children.
4. Adults.
5. Certain selected groups: children in private schools, nurses in training schools and individual families.

1. *Mothers and Young Infants.*—This study was undertaken to determine whether there was an intra-uterine transmission of scarlatinal antitoxic antibodies similar to the known transmission of diphtheria antitoxin from mother to offspring through the placental circulation.

Table 1 shows the similarity in the scarlatinal antitoxic immunity of mother and infant. The infant usually retains its immunity up to the fifth or sixth month of life, and then begins to lose it. Occasionally, an infant is seen who does not lose the antitoxic immunity until he approaches the end of the first year.

The positive reactions in young infants are much smaller and less intense than similar reactions in older children. We have noted the same phenomenon with the Schick test in young infants. The slight reactions are probably due to the limited ability of the skin of young infants to respond to inflammatory stimuli. It is due either to the slow development of this mechanism in the skin, which appears to some extent only after the infants have become a few months old, or to a natural defense mechanism with which Nature endows the young infants to protect them at the most tender period of their lives. The first explanation is probably the more correct one.

In two groups of new-born infants the Author examined specimens of blood obtained from the umbilical cord of the placenta. In one group, five of the six specimens showed the presence of antitoxin. In another group, four out of six specimens also had antitoxin. In this group the mothers and infants had also received the Dick test. In each instance in which antitoxin was present in the placental blood, the mother and infant gave a negative reaction. In the two cases in which no antitoxin could be detected, both mother and infant gave positive Dick reactions. Scarlet fever is thus the second disease, the first one being diphtheria, in which a definite placental transmission of antitoxic antibodies has been demonstrated. This is important additional evidence that we are probably dealing with similar placental transmission of antibodies against other infectious diseases, such as measles and poliomyelitis.

TABLE 1.

THE DICK TEST IN MOTHERS AND INFANTS SHOWING MATERNAL TRANSMISSION OF SCARLATINAL ANTITOXIC ANTIBODIES

0-1 Month	1-2 Months	2-3 Months	3-4 Months	4-5 Months	5-6 Months	6-7 Months
14 days .... — Mother ... —	5 weeks .... — Mother .... —	2 months ... + Mother .... +	2 3.5 months .. — Mother .... —	5 months ... — Mother .... —	5.5 months .. ⩲ Mother .... —	7 months ... ⩲ Mother .... —
23 days .... — Mother .... —	5 weeks .... + Mother .... —	2.5 months .. — Mother .... —	3 4 weeks .... — Mother .... —	2 5 months ... ± Mother .... —	6 months ... + Mother .... —	
26 days .... — Mother .... —	6 weeks .... — Mother .... —					
28 days .... — Mother .... —	2 7 weeks .... + Mother .... +					

7-8 Months	8-9 Months	9-10 Months	10-11 Months	11-12 Months	12-18 Months	18-24 Months
7.5 months .. ⩲ Mother .... —	9 months ... — Mother .... —	10 months .. ± Mother .... —	11 months .. + Mother .... +	12 months .. ⩲ Mother .... —	13 months .. + Mother .... —	19 months .. + Mother .... —
*2 8 months ... + Mother .... —						22 months .. + Mother .... +

* Indicates two similar groups observed; +, ± and ⩲ indicate varying degrees of positive reaction; — indicates a negative reaction.

2. *Age Groups.*—More than 4,500 persons who received the Dick test are classified by age groups in Table 2. These tests were made on children and adults in various institutions and in two public schools.

It is interesting to note in Table 2 the susceptibility to scarlet fever of individuals of different age groups. This closely resembles the susceptibility to diphtheria as determined with the Schick test. Other factors, however, besides age, play a significant part in determining the susceptibility of different persons to scarlet fever. These factors we have also found to be important in connection with the susceptibility to diphtheria.

TABLE 2.

THE DICK TEST AT DIFFERENT AGE GROUPS

Age	Total Tested	Dick Positive	Dick Negative	Per Cent. Dick Positive
0- 6 months	29	13	16	44.8
6-12 months	42	27	15	64.2
1- 2 years	123	87	36	70.7
2- 3 years	140	95	45	67.8
3- 4 years	207	123	84	59.4
4- 5 years	237	110	127	46.4
5-10 years	1,475	522	953	35.4
10-15 years	1,690	430	1,260	25.4
15-20 years	285	75	210	26.3
20 years up	342	61	281	17.9
Total	4,570	1,543	3,027	34.4

Table 3 shows the very high degree of susceptibility to scarlet fever among children of the more well-to-do classes in our population.

3. *Social Groups.*—Table 3 shows the results with the Dick test in two private schools. The average of positive Dick reactions among these 320 children was over 83 per cent. Even in the group between 11 and 15 years of age, the positive reactors were more than 81 per cent. This explains the high incidence of scarlet fever, once it breaks out in a school of this kind. At the Riverside Country School there were, during the past winter

TABLE 3.

THE DICK TEST IN TWO PRIVATE SCHOOLS (RIVERDALE COUNTRY SCHOOL AND HORACE MANN SCHOOL, COLUMBIA UNIVERSITY)

Age	Total Tested	Dick Positive	Negative Dick	Per Cent. Dick Positive
4 years	9	9	0	100.0
5 years	16	14	2	87.5
6 years	15	12	3	80.0
7 years	25	21	4	84.0
8 years	32	28	4	87.5
9 years	19	15	4	78.9
10 years	38	37	1	97.3
11-15 years	119	97	22	81.5
16-20 years	47	35	12	74.4
Total	320	268	52	83.7

and spring, twenty-three cases of scarlet fever out of a total school population of 125 boys varying in age between 10 and 17 years. Of seventy-one boys tested in this school, fifty-one gave a positive Dick reaction. Of the twenty negative reactors, six had scarlet fever, three during the recent epidemic and three in childhood. The Author found a similar high degree of susceptibility to diphtheria among the children of the more well-to-do of New York City.

Table 4 shows by contrast the susceptibility of the children in a public school in the borough of the Bronx. Only 22.07 per cent. gave a positive Dick reaction. It is interesting to note that in this school we had found 21.6 per cent. of the children positive to the Schick test.

TABLE 4.

RESULTS WITH THE DICK TEST AT PUBLIC SCHOOL NO. 4, BRONX

	Nonimmunes			Immunes			Total Tested	
Age	Positive	Combined	Total	Negative	Negative-pseudo	Total	Number Tested	Per Cent. Positive
5- 6	14	1	15	6	3	9	24	62.50
6- 7	18	1	19	15	3	18	37	51.35
7- 8	11	3	14	18	10	28	42	33.33
8- 9	16	8	24	24	20	44	68	35.29
9-10	6	6	12	31	17	48	60	20.00
10-15	20	26	46	175	137	312	358	12.85
Total	85	45	130	269	190	459	589	22.07

Schick Test for Diphtheria Susceptibility or Immunity at Public School No. 4, Bronx

Total	Shick Positive	Per Cent. Schick Positive
1,133	248	21.6

Table 5 shows the percentage of positive Dick reactors among children in various institutions that I tested in New York City, Rochester and Buffalo.

Table 6 gives the percentage of positive Dick reactors among nurses in different hospitals and among the students at Teachers College, Columbia University. It is interesting and rather significant to note the high proportion of susceptible persons in these groups of adults. This is accounted for by the fact that many of the nurses come into training schools from small communities where there has been comparatively little exposure to infection with the scarlet fever hemolytic streptococcus. The Lenox Hill Hospital, with many of its pupils from larger cities, shows the smallest percentage of positive Dick reactors. The percentage of positive Schick reactors in each of these institutions is also very high.

TABLE 5.

THE DICK REACTION IN DIFFERENT INSTITUTIONS

Institutions	Age in Years	Nonimmune			Immune			Total Tested	
		Dick Positive	Combined	Total Positive	Dick Negative	Pseudo	Total Negative	Total Tested	Per-Cent. Positive
Heckscher Foundation	2 wk.–5 yr.	39	2	41	31	1	32	73	56.1
Jewish Orphan Home, Rochester, N. Y.	8–16	10	7	17	6	15	21	38	44.6
Hillside House, Rochester, N. Y.	3–16	63	11	74	60	32	92	166	44.5
N. Y. Orphanage, Hastings, N. Y.	3–16	59	29	88	91	19	110	198	44.4
Society Prevention of Cruelty to Children, N. Y.	3–15	31	11	42	58	34	92	134	31.3
St. Mary's Orphan Asylum, Rochester, N. Y.	4–15	32	18	50	64	47	111	161	31.0
Hebrew Orphan Asylum, N. Y.	3–17	131	67	198	309	171	480	678	29.2
Leak and Watts Orphan Home, Yonkers, N. Y.	3–17	85	45	130	269	190	459	589	22.0
German Roman Catholic Orphan Home, Buffalo, N. Y	2–16	81	8	89	270	57	327	416	21.1
Total		531	198	729	1,158	566	1,724	2,453	29.3

TABLE 6.

THE DICK REACTION ON NURSES AT DIFFERENT HOSPITALS AND STUDENTS AT TEACHERS COLLEGE

Institutions	Nonimmunes			Immunes			Total Tested		Result with Schick Test
	Dick Positive	Positive Combined	Total Positive	Dick Negative	Negative pseudo	Total Negative	Total Tested	Per Cent. Dick Positive	Per Cent. Schick Positive
Students at Teachers College, Columbia Univ.	21	9	30	20	2	22	41	73.1	65.0
General Hospital, Rochester, N. Y.	7	8	15	10	2	12	27	55.5	75.0
St. Vincent's Hospital	37	5	42	28	10	38	80	52.5	55.0
Children's Hospital, Buffalo, N. Y.	7	4	11	11	1	12	23	47.8	60.0
New York Hospital	20	7	27	23	15	38	65	41.5	54.3
Lenox Hill Hospital	16	19	35	42	15	57	92	38.0	43.7
Total	108	52	160	134	45	179	328	48.7	

4. *In Family Groups.*—Table 7 gives the results with the Dick test in the families of six physicians. Some of the children and adults had a negative reaction with no history of scarlet fever. In the first family, that of Dr. T. E. W., the boy of 11 years had had scarlet fever recently, and on his return home gave the disease to the 10-year-old boy. The Dick test showed

TABLE 7.

THE DICK REACTION IN FAMILIES

Families	Age	Dick Positive	Dick Negative	Comment
1. Dr. T. E. W.	..	..	—	No history of scarlet fever
Mrs. T. E. W.	..	..	—	No history of scarlet fever
Tom	12	..	—	No history of scarlet fever
Frank	11	..	—	Scarlet fever, 44 days convalescence
Donald	10	..	—	Scarlet fever, 30 days convalescence
George	8	+	..	
Jack	6	+	..	
Kenneth	5	+	..	
Arthur	2	+	..	
2. Dr. J. B.	..	..	—	No history of scarlet fever
Mrs. J. B.	..	..	—	No history of scarlet fever
Margaret	15	±	..	
James	13	++	..	
Elizabeth	10	+	..	
Jeanne	7	..	—	No history of scarlet fever
Anne	18 m	+	..	
3. Dr. R. F.	..	..	—	No history of scarlet fever
Mrs. R. F.	..	+	..	
John	8	+	..	
Mary	5	+	..	
William	2	+	..	
4. Dr. H. E.	..	..	—	History of scarlet fever
Mrs. H. E.	..	+	..	
Ethel	22	+	..	
Robert	20	..	—	No history of scarlet fever
Haven	18	+	..	
Ruth	13	+	..	
Ralph	12	+	..	
5. Dr. S. W.	..	..	—	History of scarlet fever
Mrs. S. W.	..	..	—	No history of scarlet fever
Shirley	14	..	—	History of scarlet fever
Ethel	12	..	—	History of scarlet fever
Harmon	20 m	+	..	
6. Dr. A. Z.	..	..	—	No history of scarlet fever
Mrs. A. Z.	.	..	—	No history of scarlet fever
Joseph	4	+	..	
Diana	2	+	..	
Henry	11 m	+	..	

that the parents and the oldest boy were immune. All three gave a negative history of scarlet fever. The two convalescent boys also gave a negative reaction, while the four younger boys gave a positive reaction. The positive Dick reactors in all these families have been injected with increasing doses of scarlet fever toxin for the purpose of active immunization. Group 6 represents the Author's own family. The three children were also actively immunized with diphtheria toxin-anti-toxin at the age of 8 months. All three give a negative Schick reaction at the present time.

5. *Comparison Between Dick Test and Schick Test in the Same Person.*—Table 8 shows various groups of persons who were tested with both the Dick test and the Schick test. It is interesting to note that about two-thirds of the total number had similar reactions. At Public School 4, Bronx, the large majority was negative to both tests; in the private school and among the nurses at the hospitals, a very large proportion was positive to both tests. When the two reactions were different, there was approximately the same number in each group, with a slightly larger proportion in a group of negative Dick and positive Schick reactors. The repeated exposure to contact infection, with resultant development of natural immunity, need not, of course, have been equal in degree and frequency to both diphtheria and scarlet fever.

TABLE 8.

COMPARISON BETWEEN DICK TEST AND SCHICK TEST IN THE SAME PERSONS

School or Institution	Total Tested	Reactions Similar				Reactions Not Similar			
		Dick Positive Schick Positive		Dick Negative Schick Negative		Dick Positive Schick Negative		Dick Negative Schick Positive	
		Number Tested	Per Cent. Total Tested	Number Tested	Per Cent. Total Tested	Number Tested	Per Cent. Total Tested	Number Tested	Per Cent. Total Tested
Horace Mann School, Columbia College	144	107	74.3	7	4.8	15	10.4	15	10.4
Lenox Hill Hospital, nurses	23	11	47.8	0	0	2	8.6	10	43.4
General Hospital, Rochester, N. Y., nurses	23	11	47.0	0	0	2	8.6	10	43.4
Children's Hospital, Buffalo, N. Y., nurses	23	11	35.0	5	25.0	3	15.0	5	25.0
Teachers College, Columbia University	29	10	34.4	7	24.1	4	13.7	8	27.5
New York Hospital, nurses	38	10	26.3	9	23.6	9	23.6	10	26.3
St. Vincent's Hospital, nurses	71	16	22.5	22	30.9	23	32.3	10	14.4
Public School 4, Bronx	219	13	5.9	147	67.1	24	10.9	35	15.9
Total	567	185		197		82		103	
Per Cent. of total tested			32.6		34.7		14.4		18.1

RESULTS WITH THE DICK TEST IN ACUTE AND CONVALESCENT CASES OF SCARLET FEVER

During the last three months we have given the Dick test to all scarlet fever patients admitted to the Willard Parker Hospital. Most of the patients were tested on admission, and subsequently at intervals of several days. The results have been very striking, and afford good evidence of the value and reliability of the Dick test as an index of susceptibility or immunity to scarlet fever.

These observations are shown in Tables 9, 10 and 11.

1. *Summary of Results in Scarlet Fever.*—Table 9 gives a summary of the results with the Dick test in acute and convalescent cases of scarlet fever. In the first part of the table, we note that every one of the 141 patients tested during the first five days of illness had a positive reaction. The degree of reaction varied, most of the patients giving a + or ± reaction. The presence of a + + reaction after the third day of scarlet fever should raise strong doubts whether we are dealing with true scarlet fever, or at least with the usual scarlet fever virus. This doubt would be more than justified if the reaction continues strongly positive into convalescence. Among forty-nine patients admitted with a history of six days or more illness, three gave a ± and five a $\underline{\pm}$ reaction. The history of the onset of the disease in many of the patients is not always reliable.

TABLE 9.

SUMMARY OR RESULTS WITH DICK TEST IN ACUTE AND CONVALESCENT CASES OF SCARLET FEVER

1. Results of First Dick Test on Scarlet Fever Patients at Time of Admission to Hospital.

Number Days of Illness Before Dick Test	Reactions Tested					Total Tested		
	++	+	±	$\underline{\pm}$	—	Number	Dick Positive	Per Cent. Dick Positive
1-5	8	62	66	5	0	141	141	100.0
6 and over	0	0	3	5	41	49	8	16.5
Total	8	62	69	10	41	190	149	78.4

2. Total Number of Patients Receiving Dick Test During Acute Stage and Convalescence from Scarlet Fever

A. Total number tested	170
B. Total number Dick positive on admission to hospital and Dick negative during convalescence	158
C. Total number Dick positive on admission to hospital and Dick positive during convalescence	12
D. Per cent. giving negative Dick reaction during convalescence from scarlet fever	93.0

In the second part of the table are enumerated the patients who were tested on admission to the hospital and later during convalescence. Of the 170 tested, 158, or 93 per cent., gave a positive reaction earlier in the disease and a negative reaction during convalescence. There were, in addition, twelve patients who had a positive Dick reaction during convalescence. By using a

TABLE 10.

THE DICK TEST IN SCARLET FEVER PATIENTS DURING ACUTE AND CONVALESCENT STAGES OF THE DISEASE, PATIENTS SHOWING POSITIVE DICK REACTIONS DURING CONVALESCENCE FROM SCARLET FEVER

Name	Age	Date of Admission	No. of Days Ill Before Admission	Dick Tests					Comment
				First	Second	Third	Fourth	**Fifth**	
Mary F.	40	4/5	2	4/5+	4/10+	4/25+	......	......	On admission, Schultz-Charlton test with convalescent and Dochez serum moderately positive
Catherine S. ..	26	4/6	2	4/6±	4/10±	4/25±	......	......	On admission, Schultz-Charlton test with convalescent and Dochez serum moderately positive
Paula M.	5	3/16	2	3/17±	3/31—	4/10±	4/25+	......	Desquamation present
Dorothy F. ..	4	3/28	4	3/28+	3/31—	4/10±	4/25+	......	Slight desquamation on one hand; none on feet
Nema T.	28	5/17	2	5/17++	5/20+	5/22+	5/23+	5/28+	Nonscarlatinal patchy desquamation
Ray H.	7.5	4/11	2	4/13+	4/25±	4/30—	5/3±	5/10+	No desquamation. Blood taken 5/11 did not neutralize scarlet fever toxin and did not blanch rash
Tessie L.	4.5	4/6	3	4/6++	4/10++	4/25++	5/3++	5/10++	No desquamation. Blood taken 5/11 did not neutralize scarlet fever toxin and did not blanch rash
Rebecca K. ...	20	4/15	4	4/16+	4/19±	4/25±	5/3±	......	No desquamation
Margaret B. ..	2	2/15	2	......	3/6—±	3/11±	......	......	No desquamation
Isidore B.	23	3/10	2	3/11±	3/22±	4/1±	......	......	No desquamation
Mary B.	4	4/19	4	4/19+	4/25+	5/3±	5/10±	......	No desquamation
Margaret H. ..	20	4/4	5	4/4±	4/15±	4/25+	......	......	No desquamation

TABLE 11.

PATIENTS DEVELOPING SCARLET FEVER AFTER ADMISSION TO HOSPITAL

Name	Age, Years	Date of Admission	Number of Days Ill Before Admission	Dick Test on Admission		Attack of Scarlet Fever	Dick Retests During Convalescence				
				First	Second		Third	Fourth	Fifth	Sixth	Sevent
Verna B.	10	4/23	10	4/24++	4/25++	4/28	5/3+	5/4—	5/8— Ps.	5/10—Ps.	5/15—
Raymond W.	1.5	5/13	4	5/13++	5/17++	5/18	5/20+	5/22—	5/24—	5/31—	

stronger toxin dilution, it is possible, of course, to test convalescent patients, and obtain positive reactions in persons who have given a negative reaction with the standard toxin dilution. In fact, the antitoxin content of the blood of convalescents can be directly estimated in this way. The important question that has to be considered, however, is the significance of the persistent positive Dick reaction obtained with a standard toxin dilution in patients convalescent from scarlet fever.

2. *Group of Irregular Cases.*—Table 10 shows the twelve persistent positive Dick reactors. Of the twelve, the last seven enumerated had no desquamation during convalescence. Of the first four, Patient 1 and Patient 2 had shown a positive blanching test with convalescent serum and Dochez horse serum. Patient 3 had moderate desquamation, and Patient 4 slight desquamation. Patient 5 showed a patchy desquamation over both shoulders and arms, but no desquamation on the palms of the hands or the soles of the feet. Blood taken on the thirteenth day of illness, May 26, 1924, was negative for antitoxic antibodies. The interesting question has to be considered whether desquamation is a sine qua non in the diagnosis of scarlet fever.

3. *Group of Patients Developing Scarlet Fever After Admission to the Hospital.*—Table 11 shows in a very striking way the diagnostic value of the Dick test. The two patients were admitted to the Willard Parker Hospital with the diagnosis of scarlet fever. Neither one of these patients had the disease on admission, for they both developed scarlet fever five days after entering the hospital. The first patient (Verna B.) came in with a history of scarlet fever of ten days' duration. The Dick reaction on the second and the third days after admission was strongly positive (++). Such a strongly positive reaction on the twelfth and the thirteenth days of illness led the Author to make a diagnosis of "no scarlet fever." Two days later the patient developed a typical attack of the disease. The Dick reactions, made five and six days after the appearance of the rash, were slightly positive. On the tenth day the Dick reaction was negative and continued negative throughout convalescence.

The second patient (Raymond W.) also showed a strongly positive Dick test on the second and fourth days after admission to the hospital. These days would correspond to the sixth and eight days of the supposed illness, a time when the Dick reaction is usually slightly positive or negative in a true case of scarlet fever. On the fifth day after admission to the hospital the boy developed a typical scarlet fever rash. Two days later the Dick reaction was still positive. However, four days later, the reaction was only slightly positive and six days later it was negative.

A strongly positive Dick reaction noted in a case of scarlet fever after the patient has had the disease for a few days should put one on guard as to the correctness of the diagnosis. According to Pirquet, after the period of incubation of an infectious disease, during which the organisms multiply in the body and stimulate the development and gradual accumulation of antibodies, the period of invasion is ushered in by the interaction between the organisms and the antibodies that have been gradually produced. Antitoxic antibodies, present in small amounts during the early stages of scarlet fever, are sufficient to prevent the development of strong positive Dick reactions (Table 12) similar to the ones seen in normal, susceptible persons; only moderately or slightly positive reactions are noted. The Dick reaction soon becomes negative as the disease progresses into convalescence.

TABLE 12.

THE DICK REACTION IN SCARLET FEVER AND ITS RELATION TO THE SCHULTZ-CHARLTON PHENOMENON

A. The Dick Reaction in Normal Susceptible Persons and in Scarlet Fever Cases

Normal Person	Scarlet Fever			
	Period of Incubation	Period of Invasion	Period of Eruption	Period of Convalescence
++	++ changing to +	+ changing to ±	± changing to $\underline{\pm}$	$\underline{\pm}$ changing to —

++, strongly positive; +, positive; ±, slightly positive; $\underline{\pm}$, very faintly positive; —, negative.

B. The Dick Test and Schultz-Charlton Phenomenon in Scarlet Fever

	Days of Illness			
Test	1-4 Days	5-7 Days	8-10 Days	11-15 Days
Dick test	+ or ±	± or $\underline{\pm}$	$\underline{\pm}$ or —	—
Schultz-Charlton	—	— or ±	± or +	+

A definite negative Dick reaction during the first few days of the suspected attack of scarlet fever also makes the diagnosis doubtful. There is one exception, however; in the presence of an intense blush on the forearms as well as on the rest of the body, the so-called "boiled lobster rash," a moderately positive reaction may not stand out by contrast from the underlying rash and the reaction may appear negative. In some of these cases pressure on the forearm above the point of the test may make the reaction more visible.

The question will arise in many instances, when the diagnosis has been rendered doubtful by the result of the Dick test, whether it is justifiable to keep the patient in the hospital for the required thirty days. Patients who give a strongly positive reaction after admission into the scarlet fever ward can be protected after exposure by an injection of convalescent or horse antitoxic serum.

In Part B of Table 12 the relation between the Dick test and the Schultz-Charlton extinction test in scarlet fever is shown.

4. *Appearance of Old Positive Dick Reaction During Acute Stage of Scarlet Fever.*—One of the two patients shown in Table 11 (Verna B.) and another patient, a nurse, who also had given a strongly positive Dick reaction one week previous to developing scarlet fever, showed a most interesting and significant appearance of the original test at the time of the rash. The area on the forearm corresponding in size to the previous positive Dick reaction was very pale and had the typical blanched out appearance. This area was surrounded by a sharply defined ring of the scarlet fever rash, which was greatly intensified in redness when compared with the rest of the eruption on the forearm. This appearance persisted for a number of days until the rash faded. During the height of the eruption, a second Dick test was made over the site of the pale area and another one a little to one side. The reaction that developed within the pale area was very slight when compared with the second reaction beside it. Apparently, a certain amount of local cellular immunity had developed at the site of the original positive

Dick test. This immunity gave rise not only to the appearance of local pallor, which presented such a striking contrast to the rest of the blushed skin, but also to the slight positive reaction to the Dick test made over this pale area as compared with the second more strongly positive reaction on the adjacent skin. The ring of intensified rash making up the border that surrounded the pale area was probably due to the interaction between cells that were sensitized rather than protected by the minute antibody content within their substance and the toxin of the disease. These observations point to the probable local origin in the epidermis of some of the antitoxin production against scarlet fever.

One of the two patients had also received, a week previously, the initial immunizing dose of scarlet fever toxin. At the site of the injection, which was given subcutaneously in the arm, there appeared the same phenomenon during the time of the rash as was noted at the site of the Dick test. The area of pallor was larger, however, and the surrounding ring slightly wider.

5. *Group of Scarlet Fever Cases Showing Negative Dick Reactions During Convalescence (Regular Group).*—Table 13 shows twenty-seven scarlet fever patients who were Dick positive on admission to the hospital, and subsequently gave a negative reaction during convalescence from the disease. The reaction apparently becomes negative seven to ten days after the onset of the illness. In a few patients a shorter time was noted (from four to five days). The greater number of days before the appearance of a negative Dick reaction in the last three cases is accounted for by the longer intervals between the tests in these patients.

The period during convalescence when the patients show a negative Dick reaction corresponds to the appearance of antitoxic antibodies in their circulating blood (Table 9). This can be shown by the property of the serum to neutralize scarlet fever toxin and cause the blanching of an early rash in an acute case of scarlet fever (positive Schultz-Charlton test, Table 12).

The Dick reaction became negative a little earlier in convalescence, showing that it requires a certain concentration of antitoxic antibodies before the serum will cause a good blanching in an early rash. The Schultz-Charlton test as a quantitative measure for the antitoxin content is a much cruder method than the direct or indirect demonstration of the presence or absence of these antibodies by the Dick test. The table also shows that some of the patients developed a pseudoreaction during convalesence.

The results shown in Table 13 are certainly a striking corroboration of the statement of the Dicks that the reaction is positive during the early stages of scarlet fever and negative in convalescence. Two of the patients (Mollie D. and Seymour B.) had been tested at the Hebrew Orphan Asylum from one to two weeks previous to the attack of scarlet fever. Both of these children had given a + + positive Dick reaction. Four days after developing scarlet fever, Seymour B. gave a ± reaction; five days after the onset of the illness, Mollie D. gave a ± reaction. In both these children negative reactions were noted later on during the period of convalescence.

TABLE 13.

THE DICK TEST IN SCARLET FEVER PATIENTS DURING ACUTE AND CONVALESCENT STAGES OF THE DISEASE (REGULAR GROUP), WILLARD PARKER HOSPITAL

Name	Age, Years	Date of Admission	Days Ill Before Admission	Dick Test						Dick Test Negative Day of Illness
				First	Second	Third	Fourth	Fifth	Sixth	
Sophie B.	4	5/1	2	5/1 ±	5/3 —	5/10 —	5/17 —	....	....	4
Vincent C.	7	5/14	2	5/15 ±	5/17 —	....	....	....	....	5
Alfred G.	18	5/8	3	5/8 ±	5/10 —	5/17 —	....	....	....	5
Helen M.	8	4/30	2	5/1 ±	5/3 —	5/10 —	5/17 —	....	....	5
Tom R.	21	2/26	2	2/26 +	3/1 —	3/6 —	3/22 —	3/31 —	4/25 —	6
Jacob E.	11	5/6	1	5/6 ±	5/10 —	5/17 —	....	......	....	5
Rose T.	10	5/17	3	5/17 ±	5/20 —	....	....	....	....	6
Seymour B.*	13	4/8	4	4/9 ±	4/10 —	4/25 —	5/3 —	....	....	6
Mary O'H.	6	5/19	2	5/20 +	5/22 ±	5/24 —	....	....	....	7
Elizabeth G.	2	5/15	2	5/15 +	5/17 +	5/20 —	....	......	....	7
William F.	21	5/14	1	5/15 +	5/17 ±	5/20 —	......	....	....	7
Josephine M.	28	5/17	2	5/18 +	5/20 ±	5/22 —	....	....	....	7
Elias L.	9	4/29	3	4/29 +	5/3 —	5/10 —	5/17 — ps.	....	....	7
Mildred D.	9	5/19	3	5/20 +	5/22 ±	5/24 —	....	....	....	8
Mildred W.	1½	3/19	1	3/19 +	3/27 —	4/10 —	4/25 —	5/3 — ps.	5/17 — ps.	9
Edward P.	14	5/3	2	5/4 ±	5/10 —	5/17 —	....	....	....	9
Albert B.	7	5/12	1	5/12 +	5/17 ±	5/20 —	....	....	....	9
Paul B.	15	4/17	2	4/19 +	4/25 —	5/3 —	5/10 — ps.	....	....	10
Elizabeth G.	2	5/15	5	5/15 +	5/17 +	5/20 —	....	....	....	10
Margaret H.	41	4/23	3	4/24 +	4/25 +	5/3 — ps.**	5/10 — ps.	5/17 —	....	10
Nathan L.	11	5/12	2	5/12 +	5/17 ±	5/20 —	....	....	....	10
Julius C.	10	5/15	2	5/16 +	5/20 +	5/23 —	....	....	....	10
Germain B.	12	4/17	2	4/19 +	4/25 ±	5/3 —	5/10 —	....	....	10
George S.	26	4/12	4	4/4 ±	4/10 —	4/25 —	5/3 —	....	....	12
Catherine F.	14	4/24	4	4/24 ±	4/25 ±	5/3 —	5/10 — ps.	5/17 — ps.	....	13
Florence R.	1	4/26	1	4/30 +	5/ 3 ±	5/10 — ps.	5/17 — ps.	5/20 — ps.	5/22 — ps.	15
Mollie D.†	6	4/12	2	4/14 ±	4/25 —	5/3 —	5/10 —	....	....	13

* Had a ++ Dick test one week before attack of scarlet fever.

** Indicates negative-pseudo reaction.

† Had a ++ Dick test two weeks before attack of scarlet fever.

ACTIVE IMMUNIZATION WITH SCARLET FEVER TOXIN

We have injected more than 1,400 persons who gave a positive or a positive-combined Dick reaction with scarlatinal hemolytic streptococcus toxin. To avoid the constitutional symptoms noted by the Dicks, we begin with smaller doses and give three injections at weekly intervals instead of two injections at intervals of five days. The injections are made subcutaneously or intramuscularly.

We have found it convenient and helpful to interpret the dose of the toxin injected in terms of the skin test dose. In children under 12 years of age, we inject first 100 skin test doses, then 250 and again 250; for persons over 12 years of age, first 100, then 250 and 500 skin test doses are given. Recently we have been giving 500 skin test doses as the third dose to children over 3 years of age. For adults, 1,000 skin test doses may be used for the third injection. The toxin is diluted so that each cubic centimeter represents 500 skin test doses. The initial dose is 0.2 cc.; the second dose, 0.5 cc., and the third dose 0.5 or 1 cc., depending on the age of the person. When a large group is to be injected with the same dose, a dilution of the toxin is made of such strength that 1 cc. represents 100, 250, 500 skin test doses.

The reactions noted have been, as a rule, largely local and consist of a certain amount of redness and swelling at the site of injection. The second and third doses, although from two and one-half to five times as great as the first one, are followed, as a rule, by only very slight local reactions.

In a small proportion of the injected persons constitutional symptoms have been noted. In eight children and in one adult we have seen a scarlatiniform rash, which developed from twenty-four to forty-eight hours after the first injection of 100 skin test doses. There was a slight sore throat and elevation of temperature in these subjects, but no vomiting. The second injection in this group gave no constitutional and only slight or no local symptoms. The adult had a fine desquamation of the skin on the palms of the hands after the disappearance of the rash. She gave a negative Dick retest two weeks after the second injection.

Occasionally the second and even the third injection of toxin may be followed by a rash, slight sore throat and some febrile reaction. The symptoms disappear in from thirty-six to forty-eight hours. While these constitutional reactions occur only occasionally and are not serious, being seen in persons who are unusually susceptible to the effects of the toxin, yet it would seem desirable to avoid them. For that reason we are at the present time treating the purified toxin with formaldehyde according to the method suggested by Glenny and Hopkins for the production of toxoid from diphtheria toxin. This consists in adding 0.1 per cent. liquor formaldehydi to a toxin containing from 2 to 5 mg. of aminonitrogen per 10 cc., as determined by the Van Slyke method, and allowing the toxin to remain at incubator temperature for four or five weeks. In this way we aim to obtain a modified scarlet fever toxin or toxoid that will retain its immunizing value and can be used in larger doses without the risk of producing constitutional symptoms. This work is in progress, and the results will be reported as soon as they are finished.

The Dick retest, made from one to two months after the injections of the toxin, gave a great number of pseudoreactions, making quite difficult the interpretation of the developed immunity. A control test with heated toxin was found quite essential for reading the reactions at this time. With the purified toxin, the pseudoreactions in the retest can be largely avoided and the immunity results more accurately noted.

Table 15 shows the immunity results obtained with scarlet fever toxin in three institutions. The Dick retest was made from four to five weeks after the last toxin injection. At the Hebrew Orphan Asylum, 104 of the 143 injected children, or 72.7 per cent., gave a negative or pseudoreaction. At the New York Orphanage, forty-five out of ninety-one, or 49.4 per cent., gave negative or pseudoreactions. At the Leake and Watts Orphan Home, we injected the children with four doses of the toxin, consisting of 10, 25, 100 and 250 skin test doses. This institution was one of the first in which the testing and immunization against scarlet fever was carried out. We injected very small doses of the toxin at first, so as to avoid the more pronounced constitutional reactions noted by the Dicks with larger doses of the toxin. At this institution, eighteen of the forty children retested, or 45 per cent., had negative reactions or pseudoreactions.

TABLE 15.

IMMUNITY RESULTS WITH SCARLET FEVER TOXIN

Doses given patients under 12 years: 100, 250 and 250 skin test doses at weekly intervals
Doses given patients over 12 years: 100, 250 and 500 skin test doses at weekly intervals
Dick retest after four to five weeks

Institution	Per Cent Positive Dick at Original Test	Total Re-tested	Dick Positive and Combined			Dick Negative and Pseudo	
			Number Positive As in Original Test	Number Less Strongly Positive	Per Cent. Less Strongly Positive	Number	Per Cent.
Hebrew Orphan Asylum	29.2	143	19	20	14.0	104	72.7
New York Orphanage	44.4	91	10	36	39.5	45	49.4
Leake and Watts Home	22.0	40	12	10	25.0	18	45.0
Total		274	41	66	24.0	167	61.0

Of the 274 children retested in the three institutions 167 or 61 per cent, showed that they had become immune. Most of the reactions noted were pseudoreactions. The large number of pseudoreactions at the retest indicates that a certain amount of protein sensitiveness deveolped after the toxin injections.

Conclusions

1. The Dick test is a reliable index of immunity and susceptibility to scarlet fever.

2. In conjunction with active immunization with scarlet fever toxin, it will help in solving the problem of the control of scarlet fever.

3. It serves to indicate the susceptible persons who need immediate passive immunization with scarlet fever antitoxin. The possibility of interpret-

ing the reaction within twelve hours is of great clinical value for this purpose. In our experience up to the present time seven of the positive reactors and none of the negative reactors have developed scarlet fever.

4. The Dick test helps in the diagnosis of doubtful cases of scarlet fever. A strongly positive reaction early in the disease and again late in convalesence speaks against the diagnosis of scarlet fever. A definite negative reaction during the first two days of rash should also put one on guard that he may not be dealing with scarlet fever. A negative reaction does not usually develop until the sixth to the tenth day of the disease, at a time when the rash has faded. Two children who had a strong positive reaction on admission to the hospital developed scarlet fever five days later. Both patients gave a negative reaction during convalescence.

5. Other applications of the skin test consist in studying the specific character of the toxin produced by different strains of hemolytic streptococcus. Such studies would be of value in the diagnosis of doubtful cases of scarlet fever, in the classification of clinical conditions caused by the hemolytic streptococcus but not associated with scarlatiniform rashes, and in the identification of normal and persistent carriers of the specific streptococcus of scarlet fever. Patients convalescent from scarlet fever may even be required to be free from the specific organisms before they are discharged from quarantine, if a method of accurate and rapid bacteriologic diagnosis can be established.

6. A new impulse will be given by the application of the test to the study of scarlet fever to determine whether the disease includes a single clinical entity or whether, as was long suspected, the clinical conception of scarlet fever includes a variety of diseases with similar symptoms, but not necessarily associated with the presence of the specific strains of the hemolytic streptococcus of scarlet fever.

7. Scarlet fever is a combined toxic and bacterial infection, caused by a specific hemolytic streptococcus. The immunity following an attack of scarlet fever is antitoxic and not to any extent antibacterial. This is shown by the fact that convalescent patients, who give a negative Dick reaction, may develop secondary septic complications produced by the specific streptococcus. A permanent antitoxic immunity following the disease explains the freedom from second attacks of clinical scarlet fever. It is possible, however, that subsequent infections without a rash caused by the specific streptococcus may occur in those who have recovered from an attack of scarlet fever. Such infections may also occur in those who give a negative Dick reaction.

8. The diluted toxin keeps well, and can be distributed in this form ready for the test. A control test, consisting of toxin heated at boiling temperature in a water bath for one hour, has been suggested to identify the pseudoreactions.

9. The results with the Dick test in normal persons indicate that the percentage of susceptible persons by age groups and by social status resembles closely the similar percentage of persons susceptible to diphtheria noted with the Schick test.

10. The application of the Dick test in the early stage of scarlet fever showed a positive reaction in each one of the 141 patients tested. The reactions rapidly became less strongly positive and were found negative from the seventh to the tenth day of the disease.

11. Of 170 scarlet fever patients tested during convalescence 158, or 93 per cent., gave a negative Dick reaction. There is some doubt about the diagnosis of scarlet fever in most of the remaining twelve patients, who

showed a persistent positive reaction in convalescence. Positive reactions can be brought out in scarlet fever convalescence by using a stronger dilution of the toxin (1:100). It is therefore necessary, in making comparative studies, to adhere to one standard dilution of the toxin.

12. Active immunization with scarlet fever toxin is a safe procedure, and is not to any extent associated with the development of constitutional symptoms, if the dose of the toxin is gradually increased. The amount is most conveniently increased by the skin test dose, the quantity injected representing 100, 250 and 250 skin test doses for children under 12 years, and 100, 250 and 500 skin test doses for persons over 12 years of age. The injections are given one week apart. The immunity results as shown by the Dick retest seem to be encouraging. A large proportion of the selected individuals show a pseudoreaction, indicating the necessity of adding the control test to the Dick test in checking up on the immunity results with scarlet fever toxin.

13. Purification of the toxin by the sodium chlorid and acetic acid precipitation method gives a preparation that is better for purposes of the test and active immunization, as it eliminates to a large extent the proteins causing the pseudo element in the Dick test and the local reactions, as well as the sensitization after the immunizing injections.

14. Treating the toxin with formaldehyde, to deprive it of nearly all its toxic properties without impairing its antigenic value, seems to be a possible further step in the development of the process of active immunization against scarlet fever.

15. The scarlet fever toxin is neutralized in multiple proportions by the antitoxic serum.

For bibliography see the original article.

ANTISCARLATINAL SERUM OF DUAL POTENCY

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W. H. Park and A. W. Williams

The question of the complex nature of hemolytic streptococcus antiserum has been studied since the time of Marmoreck,[1] Van deVelde[2] and others.

Protective power was early demonstrated. Antitoxic powers were also demonstrated broadly for the scarlet fever strains by Moser,[3] Savchenko[4] and others and were recently made clear cut by the work of Dochez[5] and of the Dicks.[6]

Realizing that a serum having the power to protect against the living culture, as well as to neutralize the toxin, might have advantages over a strictly antitoxic serum, we have been inoculating several of our horses by what must be called a modified Moser method. The modifications have been learned by our work on diphtheria antitoxin and antipneumococcus serum, that is, that a portective serum is obtained most easily by the repeated inoculation of the whole killed culture into the vein, and that the antitoxin serum is best obtained by the repeated inoculation of toxin subcutaneously.

We inoculated the killed whole washed cultures intravenously, and the Buchner filtrates of a six day growth in blood bouillon subcutaneously. Cultures of the filtrates showed 100 colonies of the streptococcus to the cc.

One of the horses which we inoculated in this way, No. 86, has been under treatment for nine months; the others for much shorter times.

The inoculations into this horse were started on January 15th, 1925, by giving the whole centrifuged 24-hour broth cultures intravenously. Two days later the Buchner filtrate of a six-day blood broth culture was started subcutaneously. The doses were continued on alternate days and were gradually increased. In about three months the serum began to show blanching in the Schultz-Charlton test. In five months the serum was strongly blanching, and 1 cc. neutralized 10,000 Dick skin test doses of the streptococcus toxin. At this time it showed no definite protection. After nine months 1 cc. neutralized 40,000 skin doses of toxin, and 0.05 cc. protected mice against 100 M.L.D. of very virulent living streptococci.

The clinical results with this serum have been excellent but it is impossible yet to state that they have been better than those obtained with antitoxic serum alone. Theoretically such a dual serum should not only neutralize the toxic effects of the streptococcus in scarlet fever but should reduce the number of so-called complications and sequellae of this disease.

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COMPLEXITY OF THE SCARLET FEVER TOXIN AND ANTITOXIN

A PRELIMINARY REPORT

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William H. Park and Rose Goldschmidt Spiegel

While we were carrying out the tests upon which this communication is based Kirkbride and Wheeler[1] reported that a person had been found who was susceptible to the Dochez toxin but who was not susceptible to the Dick or Williams toxin and that this person's serum neutralized the Dochez toxin in two persons but did not do so in the donor of the serum. The work of Williams had already shown not only that from certain cases of erysipelas, infected wounds and normal throats, hemolytic streptococci could be isolated that produced toxins similar to those isolated from the great majority of cases of scarlet fever, but that other strains from these sources produced toxins that varied in a greater or less degree from those produced by the ordinary scarlet fever streptococci.

In testing for the potency of scarlet fever antitoxin we noticed that an amount of horse antitoxic serum which would neutralize the toxin in a majority of children would totally fail in others. It was also noted that the antitoxic serum of different horses would occasionally vary in the ability to neutralize a toxin in the same child and finally that in some individuals in

TABLE 1

A preparation of horse antitoxin which neutralizes Dick toxin in some children and not in others

Child	Result of Dick Test	Result of Serum control	0.05 cc. Serum 1:25. Horse 35 + 5 Skin Doses Toxin	0.05 cc. Serum 1:25. Horse 39 + 5 Skin Doses Toxin
1	+	—	++	++
2	+	—	—	—

Neutralizing value of antitoxin from different horses in the same individuals

Cases	Result Dick Test (1 Skin Dose)	0.05 cc. Serum 1:25 + 5 Skin Doses Toxin		0.05 cc. Serum 1:25 + 5 Skin Doses Toxin	
		Horse 87	Horse 32	Horse 7	Horse 8
3	+	++	++	—	—
4	+	+	+	—	—
5	+	—	—	—	—

An example of a child in which the usual amount of antitoxin does not neutralize the dose of toxin but a multiple dose does

Case	Result Dick Test 1 Skin Dose	1:10 Dilution	1:100 Dilution
6	+	++	++
7	+	—	++

whom the ordinary amount of antitoxin was not effective an increased amount of antitoxin was.

These observations by Kirkbride, Williams and ourselves seemed to prove that the scarlet fever toxic filtrate produced by a single strain was not a single toxin but a group of toxins and that a person might be immune to one or more of the component toxins of a toxic broth and yet susceptible to others and that certain horses react in producing antitoxin more to one of the components and certain horses to another in much the same way as Park has shown that of two horses receiving simultaneously the same quantity of tetanus and diphtheria toxins one may develop a potent diphtheria antitoxin and a weak tetanus antitoxin while the other will do the opposite. The same difference is noted in the amount of specific and group agglutinins which develop in different animals receiving the same dose of bacterial antigen.

Our interest was aroused by these observations and we decided to note carefully the results which followed mixing individual serums from convalescent scarlet fever patients with the Williams toxin and testing the mixtures on the skin of many children. One group of 26 Dick positive children which showed no serum or protein reaction gave the results (of the test) shown in table 2.

TABLE 2

Number of Child	Dick Test 5 Skin Doses	0.01 cc. Convalescent Serum A + 5 Skin Doses	0.1 cc. Convalescent Serum A + 5 Skin Doses	0.01 cc. Convalescent Serum B + 5 Skin Doses	0.1 cc. Convalescent Serum B + 5 Skin Doses
1-13	++	—	—	—	—
14	++	±	—	+1	0
15	+	+	0	±	0
16	+	+	0	—	0
17	+1	+1	0	±	0
18-19	++	++	++	+	—
20-26	++	++	++	±	—

— = negative reaction; 0 = no test made; + = moderate reaction; +1 = a somewhat stronger reaction; ++ = marked reaction.

TABLE 3

Skin Reaction—Scarlet Toxin, 0.1 cc. of 1:4000	Skin Reaction—Erysipelas Toxin, 0.1 cc. of 1:100	Skin Reaction—Scarlet Toxin, 0.05 cc. 1:2000; Erysipelas Toxin, 0.05 cc. 1:50
+1	+	+1
++	++	++
++	+	+1

+ = moderate reaction; ++ = a marked reaction.

Horse antitoxin stimulated by the Williams strain acted like convalescent serum B, and completely neutralized this toxin as shown by the Dick test in the last nine children. In a series of routine tests carried out by Schroder, Williams and Park we noted that a certain dose of an individual convalescent serum failed to neutralize the Williams toxin in 35 but did neutralize it in 218 non-immunes. An interesting point was to explain how a partially neutralized toxin produced as marked a skin reaction as it did before the addition of the serum. A test made by combining a skin test dose of erysipelas toxin with one of scarlet toxin and injecting it intracutaneously revealed the interesting fact that adding together the skin test dose of the two toxins and injecting the combined dose intracutaneously did not increase the size or severity of the skin reaction.

SUMMARY AND CONCLUSIONS

The fact that an individual convalescent scarlet fever serum neutralizes the toxin of a certain scarlet fever streptococcus in certain susceptible persons and not in others proves that the toxic filtrate has more than one toxin and that a person may be susceptible to one component and not to another or others. We can conceive that the usual scarlet fever strain makes a toxic filtrate containing the different toxins, A, B, C, D; a less common strain A, C, D, E or B, C, D, E; strains from some septic sore throats C, D, H, I and from some erysipelas cases C, D, K, L. Antitoxins to a greater or less degree would develop in any person or animal injected with any toxic filtrate to neutralize the actual toxins present in the filtrate.

The finding that a certain convalescent serum or antitoxic horse serum will fail to neutralize a toxin for certain persons in the same quantity which is successful in others, but in larger amounts succeeds, proves that the antitoxic response is different for the different component toxins either because of the different proportions of the toxins in the filtrate or because the child or animal responds differently in degree to the stimulus of the different toxins. We have the analogy of horses receiving the same amount of diphtheria and tetanus toxin. Some produce strong antitoxin for diphtheria but weak for tetanus and others strong antitoxin for tetanus but weak antitoxin for diphtheria.

A convalescent scarlet fever serum from a single patient may fail to cause blanching in a true case of scarlet fever because the two cases are due to two strains that differ in at least one toxin and so in one induced antitoxin. It is also probable that there are strains of streptococci that have the power to produce scarlet rash producing toxins which are quite different from the standard Dick strains, so that after convalescing from infections due to one strain a second infection might occur from the other strain. Every year a few cases sent into the hospital with the diagnosis of scarlet fever, after recovery develop a second attack. We have been inclined to think that the first attack was not scarlet fever while, if we had made a culture, we possiblv would have found a strain of streptococci that made different toxins from the usual cases. If this assumption is true a case of scarlet fever at the onset might give a negative Dick test to the Dick strain toxin and its serum would then neutralize the standard toxin and blanch the rash of an ordinary case of scarlet fever. The point is that it is not safe at the present time to limit the diagnosis of scarlet fever to cases due to the usual strains making the standard group of toxins. At most the exceptional strains cause only a small minority of the cases of scarlet fever. Cases that do not respond to the serum should be studied carefully to determine whether their strains are typical ones. It is possible that in these a polyvalent antitoxin serum is indicated or it may simply be that the scarlet fever streptococci or other streptococci or other micro-organisms have invaded the deeper tissues and are beyond the help of antitoxins. Further investigation is necessary to determine the value of a bactericidal serum in these cases.

REFERENCE

[1]Kirkbride and Wheeler: Proc. Soc. Exper. Biol. and Med., November, 1924, 22, 85.

HEMOLYTIC STREPTOCOCCI IN SCARLET FEVER AND ERYSIPELAS (SEROLOGIC RELATIONSHIPS)

Published in The Archives of Pathology and Laboratory Medicine, 1927,
Vol. IV, pp. 481-484.

By Anna W. Williams and Caroline R. Gurley
Assisted by M. Carmichael, F. Berman, B. Kolchin, D. Behner

Summary of Work

More than 300 case strains of hemolytic streptococci from scarlatina, erysipelas and other sources have been studied in whole or in part as to their serologic relationships. A more intensive comparative study has been made of about 150 of these strains, the results from which are summarized in two tables.

Some strains have given evidence of splitting into coarse and fine (rough and smooth) colonies, subcultures from which may give agglutinative reactions differing from the original strain. This phenomenon could explain at least some of the irregularities reported in studies of this group of bacteria.

The table shows the following results:

1. (a) From early stages of scarlatinal cases strains of hemolytic streptococci have been obtained, most of which by absorption of agglutinins fall into one of three groups and one sub-group.

(b) Accepting Griffith's Type I and Type II as distinct agglutinative groups we describe four agglutinative types and one sub-type. Type I—the same as Griffith's Type I, our type strain is 2.^{2} Only 4 strains of ours fell in this group. Including Griffith's type strain we have five strains from scarlatina and one from mastoiditis in this group. It is dominant over our strains that fall in our 55^4 group which we had earlier designated as Type I.

Type sub I. Our type strain is 55^4. Most of our strains fall in this group. As Griffith says that our 55^4 falls in his Type III group, his Type III must be considered Type Sub. I. Two Dochez strains and two Dick strains sent to us belong here.

Type II. Working with Griffith's Type II strain we have found no strain in New York City that falls in this group.

Type III. All of our scarlatina strains that belong to anginosus by carbohydrate fermentation—9 strains—fall together by absorption of agglutinins. 57^2 is the type strain of this group.

Type IV. A comparatively large group of strains—17—are of this type. The most important fact concerning this group is that its members cross absorb with strains of Group I,—16 strains—from erysipelas. The type strains is 28^1.

Strains of hemolytic streptococci from erysipelas fall into one large group, Type I, a small group, Type II and a few strains not yet grouped. Most of Birkaug's strains fall into the larger group. The 3 strains of Type II belong to the subacidus group by carbohydrate fermentation.

Strains from all of these groups both from scarlatina and from erysipelas produce a toxin that may be neutralized in some human beings by both scarlatinal convalescent serum and by our Type sub. I antitoxic horse serum.

TABLE I

RELATIONSHIP OF STRAINS OF HEMOLYTIC STREPTOCOCCI TO CARBOHYDRATE REACTIONS AND AGGLUTININ ABSORPTIONS

		Carbohydrate	Reactions				Agglutinin	Absorptions							
Source	No. of Cases	Lactose	Manitol	Salicin	Inulin	Holman's Types	Type I (22)	Type Sub. I (55)	Type II Grif.	Type III (57)	Type IV (28)		Type Ery. I (C^3)	Type Ery. II (B^3)	No type yet found
Scarlatina	77	+	—	+	—	Pyogenes	5	38	1	..	17	=	(17)	..	16
Scarlatine	9	+	—	—	—	Anginosus	..	..	..	9	..		..	..	..
Scarlatina	3	—	+	+	—	Infrequens	..	..	..	..	..		..	..	3
Erysipelas	22	+	—	+	—	Pyogenes	..	..	..	..	(16)	=	16	..	6
Erysipelas	4	—	—	—	—	Subacidus	..	..	..	..	..		..	4	..
Other sources	16	+	—	+	—	Pyogenes	1	3	..	..	1	=	(1)	..	11

A type sub. I horse antiserum which is strongly protective against the subtype I strains (whole culture), has not protected against the whole culture of the type strain 28[1] of Type IV group.

CONCLUSIONS

Scarlatinal rash is merely a symptom produced by the toxins[1] of a large group of hemolytic streptococci, certain members of which may cause erysipelas. These members, while producing a toxin similar to that of all strains from scarlatina, may stimulate the production of an antiserum against the whole culture different from that produced by strains belonging to other types. Erysipelas, therefore, may require a special *antibacterial* serum while any type of antitoxic serum (stimulated by scarlatinal or other hemolytic streptococcal exotoxin) may combat the exotoxic element in at least certain cases of this disease.

THE PURIFICATION OF THE STREPTOCOCCUS ANTITOXIN FOR SCARLET FEVER

EDWIN J. BANZHAF

The method herein given is the same as used by us in experimental purification of diphtheria and tetanus antitoxins since 1912.

To the citrated plasma 0.8% of an equal volume of trikresol and ether is added. A half volume of water is then used to dilute the trikresolized plasma. Saturated solution of ammonium sulphate is added up to 25%. Thus to every 75 cc. of diluted plasma 25 cc. of saturated solution ammonium sulphate is required. A slight precipitation is observed at this point. The whole is heated rapidly up to 57° C. and held at this temperature for one and one-half hours. It is then removed and filtered hot. During the heating, considerable more precipitation is formed. This precipitate, which contains only the antitoxin held in the remaining fluid of the moist precipitate, can be discarded or it can be taken up with water and reprecipitated with ammonium sulphate and the resulting filtrate added to the first.

The clear filtrate from the heated mixture is brought up to 46% saturated solution ammonium sulphate. This will precipitate all the antitoxin, and after standing over night is filtered through hardened papers or muslin cloths. Press to remove all excess of fluid and dialyze in parchment bags in running water. Dialysis is continued for eight days or until the solution is practically free from ammonium sulphate. After dialysis 0.8% salt is added and 0.8% of an equal volume of trikresol and ether. It is then stored in a cold room for at least two weeks, preferably a month. It can then be pulped and Berkefeld filtered.

CONVALESCENT WHOLE BLOOD, PLASMA AND SERUM IN PROPHYLAXIS OF MEASLES

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Abraham Zingher

The use of convalescent serum in the prophylaxis of measles was a logical sequel to the application of human convalescent serums in preventing and curing other infectious diseases for which the causative agents, as in measles, have not yet been definitely isolated and identified. Scarlet fever and poliomyelitis are additional examples of infections in which the serum of convalescents and of recovered patients has been successfully used in prophylaxis as well as in therapy.

The serums of adults who have had these infections in childhood also contain antibodies, although they are present in a lower concentration. Such antibodies are transmitted from the mother to the offspring through the placenta. This is shown by the temporary protection of many infants under 6 months of age against different infectious diseases, such as measles, scarlet fever and poliomyelitis. In the transmitted immunity to diphtheria there is even a close quantitative parallelism in the antitoxin content of the mother's blood and that of the new-born infant.

The presence of these protective antibodies in the parents' serum is extremely important, as it enables us to utilize a source of serum supply that is universal and readily available, whereas with convalescent serum we are dependent on a limited number of donors. In this connection it may be mentioned that the Writer strongly recommended, during the epidemic of poliomyelitis in 1916, that the blood serum obtained from parents be injected in doses of from 30 to 60 cc. into young and susceptible children, so as to reestablish a temporary passive immunity during the period of epidemic.

Historical

The first published results on the use of convalescent measles serum were by Nicolle and Conseil, and appeared in 1918. These observers in 1916 succeeded in protecting a child after exposure to measles. In 1916, Park and Zingher injected forty-eight exposed children with convalescent measles serum at the Metropolitan Hospital on Welfare Island. Twenty-eight of these children received doses varying from 1.5 to 4 cc. and twenty received doses of 8 cc. In the first group, six children developed measles; one, two days; one, seven days; one, eight days; one, fifteen days; one seventeen days, and one, twenty-five days after the serum injection. In the second group, none developed measles. These results were not published.

In 1918, Richardson and Connor of Boston reported the successful use of convalescent serum in a small group of exposed children. In 1919 Degkwitz, working in Pfaundler's clinic in Munich, began a systematic series of observations with convalescent serum in the prophylaxis of measles. He subsequently organized the work on a broad scale in Munich and up to the present time has injected more than 1,000 children with convalescent serum. He was followed by various observers in Germany, Hungary, Italy, Spain, South America and the United States.

TABLE 1.

DEATHS FROM MEASLES IN NEW YORK CITY FOR YEAR 1922

Age in Years	Total Deaths	Total Deaths in Each Group	Per Cent. of Total Deaths in Each Group	Per Cent. of Deaths Under 5 Years
Under 1	236			
From 1 to 2	410			
Total		646	66.1	93.4
From 2 to 3	160			
From 3 to 4	64			
From 4 to 5	43			
Total		267	27.3	
From 5 to 9	54			
10 and over	10			
Total		64	6.6	
Total		977		

Total deaths from Jan. 1 to July 30, 1922 .... 935 (95.7%)
Total deaths from July 1 to Dec. 31, 1922 .... 42 (4.3%)

Donors and Methods of Obtaining Blood

The donors should be healthy persons, preferably adults, who are free from syphilis and tuberculosis. When adult donors are not available, it may be justified to take, under special conditions, a small amount of blood from children over 10 years of age. It is difficult, however, to see the advisability of the recommendation made by Degkwitz, that as much as 60 cc. of blood should be taken from children under the age of 5 years. I am afraid that bleeding such young children will get this method sooner or later into disrepute. Parents might be willing to allow a small amount of blood to be taken from convalescent children or from brothers and sisters who have had measles previously, for use in the same family; but most parents would decidedly object to having children under 5 years or even under 10 years of age bled for general serum distribution.

From adult donors, about 500 or 600 cc. of blood could be readily obtained. A second bleeding from the same donor could be obtained two or three weeks later, if further observations show that these late serums are fairly equivalent to convalescent serums in concentration of antibodies. Suitable compensation should be made to the donor for the blood withdrawn.

The blood should be collected under sterile precautions by allowing it to flow into a 500 or 1,000 cc. bottle. The container is closed with a double perforated stopcock, through which pass two pieces of glass tubing. To one is attached a piece of rubber tubing and a large bore (16 gage) needle, similar to those generally used in blood transfusions. The other piece of glass tubing is plugged with a little cotton, and to it is attached a piece of rubber tubing, which serves the purpose of producing a partial vacuum in the bottle by means of suction. The entire apparatus should be wrapped and sterilized. The blood can be collected and allowed to clot, and the serum subsequently drawn off, or it may be kept fluid by the addition of 0.5 per cent. sodium citrate and the supernatant plasma afterward decanted. One volume of 5 per cent. solution of sodium citrate to every nine volumes of blood is previously placed in the bottle. The decanted serum or plasma is subsequently tested for sterility, pooled with the serums of two other donors, and divided into individual ampules containing 3 cc. Degkwitz considers 2.5 cc. as one immunizing unit. For the better preservation of the serum, it has been recommended to dry it in the ampules in vacuo, and then dissolve the residue with salt solution, before use. The fluid serum has been found to retain its protective properties for six months.

Optimum Time for Bleeding Donors; Pooled Serum

According to Degkwitz, the best time for bleeding convalescent donors is from the sixth to the tenth day after the temperature has reached normal. Blood serum obtained from persons who recovered from measles several months or even several years previously has also been used with success. The dose, however, should be larger.

The Wassermann test is made on the serums unless the donors have been previously tested. Three different serums should be pooled. It is possible that an occasional convalescent donor may be a poor antibody producer, just as certain horses are poor antitoxin producers; the pooling, however, will minimize the danger of using ineffective serums.

Dose of Serum and Period of Exposure

The dose recommended by Degkwitz is 2.5 cc., or 1 immunizing unit, for children who have been exposed for not more than four days; 5 cc., or 2 units, for children exposed for from five to six days, and 7.5 cc., or 3 units, for children exposed seven days to infection with the measles virus. According to the same observer, the injection of serum on the eighth day of exposure and later will not prevent the development of the disease, nor will it modify the character of the symptoms. Our own experience and that of some of the French observers does not bear out this statement. The disease can be prevented in some of the patients, and is distinctly modified in some of the others, when the serum is injected as late as the eighth or ninth day of exposure. The serum is injected intramuscularly into the arm, thigh or gluteal region.

The dose recommended is for a child 3 years of age. For older children the dose should be in proportion to the age and weight of the child.

The period of exposure is calculated as follows: When the exposed children have been constantly in contact with the measles patient and the latter is seen on the first day of rash, the period of exposure is counted as four days; if seen on the second day of rash, as five days, etc. If the patient is seen at the time of the appearance of Koplik's spots and no rash, the exposure of the contacts is three days. Occasionally a patient is brought into a hospital and the rash appears on the following day or two days later. The exposure of the contacts will be one or two days.

Complete Passive Immunization

There are distinct indications for attempting to produce complete passive immunization by increasing doses of serum. As thorough and as complete protection as possible should be given to many children who are very young or feeble; children suffering from rickets, tuberculosis, diphtheria or whooping cough; those convalescing from other infectious diseases, and children who would be in immediate danger from an attack of measles, such as undernourished patients or feeding infants. In institutions, also, and in hospitals, where the paramount indication is to stamp out an outbreak of measles, the early and prompt administration of convalescent serum is strongly indicated and will often prove a life saving boon. The colder seasons of the year, with the greater danger of respiratory complications, will also often be found an indication for complete immunization.

MODIFIED MILD MEASLES

In private practice, when we are dealing with normal and healthy children, special conditions may also indicate the advisability of producing a complete but temporary protection.

Every clinician realizes, however, that it would be much better under ordinary conditions if our efforts should result in a permanent immunity of the children with the least risk involved. Such a permanent protection can be obtained by an attack of measles. If the disease can be modified, however, so that a mild attack of measles will develop, we shall have accomplished our aim. Such modified measles can often be produced if children are injected from the fifth to the tenth day after exposure with 2.5 cc. of convalescent serum, or with 5 cc. of serum from brothers and sisters who have recovered from measles some time previously, or with 10 cc. of adult serum. The principle involved in this method has been recommended by Debré and Ravina, and it would appear to be a more reasonable and logical procedure in private practice than the attempt to produce a complete but short immunization. My own observations also indicate that a peculiarly modified, mild form of measles can be obtained when small doses of convalescent serum are injected as late as the eighth day of exposure.

The modified form of measles is characterized by a moderate rise of temperature lasting for one or two days, and by a curious rash, scarcely resembling ordinary measles, which is scattered over face and body, and to a less extent over the extremities. The individual macules are about the size of the head of a pin, occasionally surmounted by a tiny vesicle. At times there are larger macules scattered between the small ones. Conjunctivitis, coryza and Koplik's spots are frequently absent; when present, these symptoms are very mild. The clinical picture is one of a mild form of measles as compared with the type usually seen. According to various observers, complications are very rare. In the group of cases that came under the Writer's observation, no complications were noted. Degkwitz also states that the modified form is not very infectious. This is probably on account of the slight or absent catarrhal symptoms with the lessened possibility of droplet infection.

Convalescent serum, if always available, would be most suitable for the purpose of modifying the attack of measles. Practically, however, convalescent serum will frequently not be obtainable. Under all conditions, the blood of brothers and sisters or of parents can nearly always be obtained. The serum or plasma does not have to be first separated and then injected. With an ordinary 10 or 20 Record or Luer syringe the blood is simply withdrawn and immediately injected intramuscularly. To enable the physician to work without haste, from 1 to 2 c.c. of a 5 per cent. solution of sodium citrate can be drawn into the syringe before the blood is taken from the donor. The whole blood is injected into the thigh muscles or into the gluteal region according to the intramuscular method that the Author recommended for the treatment of scarlet fever with convalescent whole blood. When whole blood is used, instead of serum, double the amount recommended for serum should be injected.

Duration of Immunity Conferred by Convalescent Serum

The duration of passive immunity produced by injecting convalescent serum in the doses recommended during the first few days after exposure is rather short, not lasting more than from four to five weeks. When convalescent serum is injected on the sixth day or later, a mixed type of immunity is produced, which lasts for from two to three months. If a modified attack of measles develops, the immunity is probably permanent.

The reports of different observers indicate that the passive immunity will last only from thirty to forty-five days. Following a second exposure, one of our patients developed typical measles forty-two days after a protective dose of serum.

Table 2 gives the dosage of convalescent serum, or serum from recovered patients (brothers and sisters) and from adults. It also indicates the type of immunity in which the protection is complete, and the type of disease in which the protection is incomplete.

Central Serum Stations

The New York City Department of Health is offering at the present time to practicing physicians of the city a limited amount of convalescent plasma in individual vials containing 3 and 5 cc. A request is made that the physicians report to the department careful observations of the results obtained.

Personal Observations with Convalescent Whole Blood and Serum

The ease and simplicity of using citrated whole blood in hospital work first induced the Writer to immunize the exposed children in this way. The more recent work was carried out with convalescent serum or plasma.

The donors were from five to fifteen days convalescent patients. In some instances we used the blood of individual donors; in others, the pooled blood, plasma or serum of several donors.

The total number of exposed persons injected was 160. Tables 3 and 4 give a summary of the different groups. Each group represents one or more individuals, who were exposed at the same time either in hospital wards at the Willard Parker Hospital, in institutions or in private homes.

TABLE 2

Measles Prophylaxis

Days of exposure		1	2	3	4	5	6	7	8	9	10	11	12
Onset of clinical symptoms in measles		Catarrhal		Kop-lik spots	1st day of rash								
Amount of serum for complete protection	Convalescent	2.5 to 3 c.c.				5 c.c.		7.5 c.c.					
	Recovered cases (brothers and sisters)	5 to 6 c.c.				7.5 c.c.		10 c.c.					
	Adults (parents)	10 c.c.				15 c.c.		20 c.c.					
Type of immunity in complete protection		Passive				Mixed				None			
Amount of serum for incomplete protection	Convalescent	1.5 c.c.					2.5 c.c.						
	Recovered cases (brothers and sisters)	2.5 c.c.					5 c.c.						
	Adults (parents)	5 c.c.					10 c.c.						
Type of measles in incomplete protection		Modified										Typical	

GROUPS OF CASES IN WHICH MEASLES CONVALESCENT BLOOD, PLASMA OR SERUM WAS INJECTED

GROUP 1.—Five children were exposed in the diphtheria pavillion, May 24. Eight days later each child was injected with 20 cc. of convalescent whole blood from a donor five days convalescent. Two of the five children developed modified measles, one ten days after receiving the injection and after an incubation period of eighteen days; the other one fourteen days after the injection and after an incubation period of twenty-two days. The rash consisted of a few scattered macules, the size of the head of a pin. In the first child the rash was limited to the face; in the second one it was also present on the trunk, but not on the extremities. Neither one of the children showed catarrhal symptoms, conjunctivitis or Koplik's spots. There was slight elevation of temperature for two days. The clinical picture was that of a mild atypical form of the disease, which could not have been identified as measles except for the history of exposure. These cases indicate that measles developing in children who receive convalscent serum eight days or more after exposure may show a modified form. The other three children of this group were protected. One child, aged 3 years, exposed at the same time, received no convalescent blood, and developed typical measles fifteen days after exposure.

GROUP 2.—This child was in the same cubicle with the control just mentioned. She was injected on the day after the rash appeared (the fifth day of exposure) and was protected.

GROUPS 3 and 4.—Ten children were exposed in one of the scarlet fever wards. May 2, the fourth day of exposure, eight of the ten children, varying in age between 2 and 5 years, were each injected with 20 cc. of convalescent blood. These children were protected. The supply of convalescent blood being exhausted, the remaining two children were not injected until May 5, the seventh day of exposure. One of these a child, aged 4, an older brother of a boy, aged 2, injected in the group of eight children, developed a typical rash on May 25, twenty days after the injection of blood and twenty-seven days after exposure. He had a modified rash and mild catarrhal symptoms.

GROUP 5.—Four children were exposed in one of the wards of the reception hospital. Each of the children was injected with 20 cc. of convalescent blood on the seventh day of exposure. No measles developed. A fifth child, aged 5 years, was not injected on account of a questionable history of measles, and developed a typical measles attack on the thirteenth day of exposure.

GROUP 6.—Four children were exposed in one of the diphtheria wards to a patient with measles who developed a rash on the day after admission to the hospital. On the following day, the second day of exposure, each of the four children was injected with 20 cc. of convalescent blood. No measles developed.

GROUP 7.—This child was in a separate room in the scarlet fever pavilion and was exposed to a second child who developed a typical measles rash four days after admission to the hospital. Twenty cubic centimeters of convalescent blood was injected and the child was protected.

GROUP 8.—A child aged 2 years, injected on the eighth day of exposure with 20 c.c. convalescent blood, was protected. The child was in a separate room in the scarlet fever pavilion, and was exposed to a patient who had developed a measles rash four days previously.

TABLE 3.

CONVALESCENT WHOLE BLOOD, PLASMA AND SERUM IN THE PROPHYLAXIS OF MEASLES

	Patients Injected								Controls	
Group	Number Injected	Age, Years	Convalescent Whole Blood	Convalescent Serum or Plasma	Days of Exposure	Number Developed Measles	Period of Incubation	Type of Rash	Total Number and Age	Number That Developed Measles Period of Incubation and Type
1	5	2, 2, 3, 5, 6	20 c.c.	......	8	2	18 days 22 days	Moderate	1 (3 yrs.)	1 (15 days) Typical
2	1	1½	20 c.c.	......	5	0	......	....	....	....
3	8	2, 2, 2, 2½, 2½, 4, 5, 5	20 c.c.	......	4	0	......	....	....	....
4	2	4, 4	20 c.c.	......	7	1	27 days	Typical	....	....
5	4	2, 2½, 2½, 5	20 c.c.	......	7	0	......	....	1 (5 yrs.)	1 (13 days) Typical
6	4	1½, 2, 3, 4	20 c.c.	......	2	0	......	....	....	....
7	1	3	20 c.c.	......	4	0	......	....	....	....
8	1	2	20 c.c.	......	8	0	......	....	....	....
9	2	3, 6	10 c.c.	......	3	0	......	....	....	....
10	2	22, 25	15 c.c.	......	6	0		....	....	....
11	1	1½	10 c.c.	......	4	0	......	....	....	....
12	2	4, 6	......	10 c.c. S.	5	0		....	....	....
13	3	2, 3, 4	......	5 c.c. S.	4	1	(42 days)* 14 days	Typical	....	....
14	1	3 days	......	5 c.c. S.	3	0	......	Moderate	....	....
15	1	2½	......	5 c.c. S.	8	1	16 days	....	3 (1 to 3 years)	2 (12 days) Typ. 1 (13 days) Typ.
16	4	6 m., 6 m., 7 m., 10 m.	......	2.5 c.c. S.	6	0	......			
17	30	1 to 5 yrs.	......	2.5 c.c. S.	6-8	4	16, 17, 17, 20	Moderate	....	....
18	5	1½, 2, 2, 2, 2½	......	3.0 c.c. P.	1-4	0	......	....	....	....
19	4	3, 5, 5, 8	......	3.0 c.c. P.	1	0	......	Typical	....	....
20	5	4, 5, 8, 8, 9	......	3.0 c.c. P.	4	1	14 days	....	....	....
21	5	2, 3, 3, 3. 7	......	3.0 c.c. P.	4	0	......	....	....	....
22	5	6m., 7 m., 1½, 1½, 2	......	3.0 c.c. P.	5	0	......	....	....	....
23	3	1, 2, 4	......	3.0 c.c. P.	6	0	......	....	....	....
24	3	2, 2, 2½	......	3.0 c.c. P.	6	0	......			
Total......	102				...	10	......	7 Mod. 3 Typ.	5	4 Typical

* Forty-two days after the injection of serum and fourteen days after the second exposure.

Group 9.—Two children were convalescing from chicken-pox in one of the wards of the reception hospital, when they were exposed to a case of measles. On the day Koplik's spots were seen in the patient, the third day of exposure, each of the two children received 10 cc. of whole blood and both were protected.

Group 10.—Two adults, who were nurses in training at the Presbyterian Hospital, never had had measles, and were exposed to a colored maid who had developed a measles rash two days previously. Each of the two nurses received 15 cc. of whole blood on the sixth day of exposure, and both were protected.

Group 11.—On the same day the nurses were injected, there was admitted to the emergency ward of the Presbyterian Hospital a little girl with Koplik's spots and a beginning rash. A younger brother, aged 1½ years, was seen at his home the same evening and injected with 10 cc. of whole blood. The child was protected.

The following thirteen groups of children were injected with convalescent measles serum or plasma.

Group 12.—Two children convalescing from scarlet fever were exposed to a child in an adjoining crib, who developed a measles rash the day before. Each child received 10 cc. of convalescent serum on the fifth day of exposure. Both children were protected.

Group 13.—Three children were exposed in the diphtheria pavilion to a child who developed measles fifteen days after admission to the hospital. On the fourth day of exposure, the day the rash appeared, each of the three children was injected with 5 cc. of convalescent serum that had been kept in the icebox for two and one-half months. The children were protected. *One of these children was subsequently exposed to another case of measles twenty-eight days after the serum injection, and following an incubation period of fourteen days he developed a typical attack of measles.*

Group 14.—At the request of the assistant resident physician, the Writer injected a 3-day old colored baby with 5 cc. of convalescent serum. The mother had given birth to the baby on the day the measles rash appeared. The baby remained free from measles.

Groups 15 and 16.—Five children were injected, December 26, in the service of Dr. Schick at Mount Sinai Hospital. They had been exposed to a child who had developed measles two days previously. The four young children, varying in ages from 6 to 10 months, were emaciated feeding cases in which measles would have been a dangerous complication. Each child received 25 cc. of convalescent serum on the sixth day of exposure. Another child, aged 2½ years, was injected two days later (eighth day of exposure) with 5 cc. of serum. The four children injected on the sixth day of exposure were protected. The fifth child, injected on the eighth day, developed a modified mild form of measles sixteen days after exposure. Three other children between 2 and 5 years of age exposed at the same time were not injected. Two of the three children developed typical measles after the usual incubation period.

Group 17.—This group was seen at the Society for the Prevention of Cruelty to Children. It consisted of thirty children under 5 years of age, who were exposed for from six to eight days before receiving convalescent serum. The dose for each child was 2.5 cc. Of the thirty children, four injected on the eighth day of exposure developed a mild, modified form of measles.

Group 18.—These five children were in the diphtheria pavilion and were injected each with 3 cc. of convalescent plasma. They had been admitted to the hospital ward on different days, and were exposed as follows: one child had one day, one had two days and the other three had four days of exposure to a measles patient (Group 13) who had been injected forty-two days previously with convalescent serum. The five children were protected.

Group 19.—Four children were exposed in a small ward at the Ruptured and Crippled Hospital to a child that had been admitted to the hospital the day before. The rash appeared twenty-four hours after admission. Each of the exposed children was injected with 3 cc. of convalescent plasma. No measles developed.

TABLE 4.

SUMMARY OF RESULTS WITH CONVALESCENT WHOLE BLOOD, PLASMA AND SERUM

Days of Exposure to Measles	Total Number Injected	Number That Developed Measles	Period of Incubation Days	Type of Measles
1	5	0	......	....
2	5	0	......	....
3	3	0	......	....
4	26	2	42	Typical
			14	Typical
5	8	0	......	....
6	17	0	......	....
7	11	1	27	Typical
8	27	7	16-22	Modified
Total	102	10		7 Modified 3 Typical
Controls	5	4	12-15	Typical

Group 20.—Five children in the diphtheria pavilion were injected with 3 cc. of convalescent plasma on the fourth day of exposure. One of the five children developed a fairly typical but moderate rash fourteen days after exposure and ten days after the plasma injection. The elevated temperature persisted for two days. There was some coryza, slight cough and Koplik's spots. The child made a rather rapid recovery.

Groups 21 and 22.—Ten children were exposed in one of the diphtheria wards to two cases of measles. The children in Group 21 received the convalescent plasma on the fourth day of exposure, and those in Group 22 on the fifth day of exposure. No measles developed.

Group 23.—Three children in two families received the convalescent plasma on the sixth day of exposure. The children were protected.

Group 24.—Three children were injected in one of the wards of the pediatric service of Bellevue Hospital. They had been exposed for six days. No measles developed.

Table 5 shows the results with convalescent plasma in two institutions in which measles had developed. It is interesting to note at the Israel Orphan Home the three individual groups of children. Each group was separated from the others, occupied a different floor, and received a dif-

TABLE 5.

EFFECT OF PERIOD OF CONVALESCENCE ON EFFICIENCY OF BLOOD PLASMA IN THE PROPHYLAXIS OF MEASLES

Children Injected											Controls*			
			Convalescent Plasma				Developed Measles					Developed Measles		
Institution	Groups of Children	Date of Injection	No. Days Convalescent	Amount Injected, cc.	Number Children Injected	Age, Years	Period of Incubation, Days	Total	Mild	Typical	Total	Total	Mild	Typical
Israel Orphan Asylum	1st	2/17/24	Mixed 8-15	3	12	1-5	10-12	6	6	0	10	1	0	1
	2d	2/27/24	13	3	20	1-5	10-11	9	9	0	6	2	0	2
	3d	2/29/24	35	3	18	4-7	10-13	7	3	4	17	7	3	4
Total					50	..	....	22	18	4	33	10	3	7
Masters Day Nursery		2/21/24	8	3	8	2-5	17-19	8	5	0	4	3	0	3
Total in both institutions					58	..	....	30	23	4	37	13	3	10

* In some of the controls as well as in some of the injected children at the Israel Orphans Home there was a doubtful history of measles. The parents will at times give a definite history that the child has had measles when the child has really not had the disease, so as to insure its admission into an institution.

ferent preparation of convalescent plasma. In Group 1, a preparation was used consisting of a mixture of three plasmas obtained from the eighth to the fifteenth day of convalescence. Of twelve children injected, six developed a mild form of measles with little or no catarrhal symptoms. One child that was not injected developed typical measles. In Group 2, the plasma of a single donor, thirteen days convalescent, was used. Of twenty injected children, nine developed a mild type of measles; of six children who were not injected, two developed typical measles. In Group 3, a pooled plasma was used obtained from thirty-five convalescent donors. Of eighteen children injected, seven developed measles, three mild and four typical with characteristic catarrhal symptoms. Of seventeen children who were not injected, there were also seven who developed measles, three a mild and four a typical form.

SUMMARY OF CASES IN WHICH INJECTIONS WERE GIVEN

Of the 102 children receiving convalescent whole blood, plasma or serum, ten developed measles. Of these, seven were injected on the eighth day of exposure, and developed a modified form of measles after periods of incubation varying from sixteen to twenty-two days. One child injected on the seventh day of exposure developed typical measles twenty days later, after a period of incubation of twenty-seven days. The tenth child was protected by 5 cc. of convalescent serum for twenty-eight days, when she was exposed to a second case of measles and developed a typical attack fourteen days later, forty-two days after receiving the serum.

Of the five control children, four developed typical measles after periods of incubation varying between twelve and fifteen days.

Of fifty-eight additional children injected in two institutions, twenty-three developed a mild and modified form of measles, and three a typical form of the disease with characteristic catarrhal symptoms. In these three children, a thirty-five day convalescent plasma was used in doses of 3 cc. Of thirty-three control children who were not injected, three showed a mild form and ten a typical form of measles after the usual period of incubation.

For bibliography see the original article.

SUSCEPTIBILITY OF RABBITS TO THE VIRUS OF MEASLES

Inoculations with Nasopharyngeal Material

Published in The Journal of Infectious Diseases, 1922, Vol. XXX, pp. 86-90

M. Grund

The investigation, the results of which are reported now, was undertaken to determine whether the inoculation of nasal secretions from patients with measles would produce definite and characteristic symptoms in rabbits; it was carried on at the same time, and with material from the same patients as were used by Nevin[1] in her work on blood inoculation.

The nasopharynx of patients in the early eruptive, or pre-eruptive stage of measles was irrigated with 30 to 50 cc. normal salt solution. Cultures were made, from the material thus obtained, on blood-vitamine-agar, to establish the prevalent types of bacteria. From 5 to 10 cc. of the washings were injected into the trachea of rabbits, the animals being lightly anesthetized. In most animals this amount of fluid caused a leakage from the nose, showing that the mucous membrane of the upper air passage was thoroughly flooded with infectious material. Unfiltered washings were used in most cases, as it was believed that the usual flora of the upper respiratory tract would not cause characteristic symptoms in rabbits. Only a small number of rabbits received material passed through a Berkefeld V candle. Aerobic cultures from these filtrates were uniformly negative for the test organism (B. prodigiosus).

The majority of rabbits (table 1) gave a certain reaction, yet apparently there were among them a fairly large number of refractory individuals for, in a number of instances in this series, only one animal out of two inoculated with the same nasal washings developed symptoms, and even when both succumbed there was often considerable difference in the severity of symptoms. In susceptible animals the incubation period varied between two and seven days.

The least reliable and constant symptom seems to be the enanthem; while present in about 20% of the cases, only 5 animals showed what one might describe as good typical Koplik spots. The temperature curve also is not at all characteristic; in very few animals did the temperature go above 103°F. In some of the cases a certain relationship appeared between the temperature curve, the cutaneous symptoms and the leukocyte count; however, it was far from constant, and the fluctuations noted in daily blood counts made for one week before inoculation do not make a "leukopenia" seem a very dependable diagnostic sign in rabbits. In a number of animals a distinct polychromatophilia was noted.

Conjunctivitis and inflammation of the upper respiratory passages, in varying degrees of severity, occurred in 70% of animals; it is not always possible to eliminate "snuffles," but the appearance of coryza together with the rash, and its brief duration in most cases, point against its being an intercurrent infection. Seventy-five per cent. of the rabbits showed some form of cutaneous eruption, either a diffuse, punctuate erythema, which in 3 severe (2 fatal) cases became petechial, or, sometimes following the

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[1] J. Infect. Dis., 1921, 29, p. 429.

TABLE 1

RABBIT INOCULATIONS WITH NASOPHARYNGEAL WASHINGS

Case	Rabbit No.	Exanthem						Desquamation			Coryza			Conjunctivitis			Enanthem		Temperature Extremes	Leukopenia			Remarks
		Erythematous			Maculopapular																		
		Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset		Intensity	Day of Onset	Duration	
M19	191	+	2	2	..	..	..	..	..	..	+	9	2	..	..	..	..	..	102.2-102.8	..	..	..	
	192	+	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	102 -102	..	..	..	
M20	201	..	..	..	..	..	..	..	..	..	+	12	1	..	..	..	..	..	102.6-102.8	+	10	1	Died after 4 days; dysentery
M21	211	..	..	..	..	..	..	..	..	..	+	3	4	+	3	1	+?	6	101.3-103	+	2	1	
	212	++	2	2	..	..	..	..	..	..	+	2	2	+	2	1	..	..	102 -105	++	3	1	
M22	221	+	2	4	+	4	2	+	5	2	+	4	2	+	4	1	..	..	100.8-102.4	+	3	1	Died after 4 days; broncho-pneumonia
	222	..	..	..	..	..	..	+	6	3	..	..	..	..	..	..	..	..	99.8-102.2	+	2	2	
M23	231	+	2	4	..	..	..	+	5	6	+	3	1	..	..	..	..	..	102 -104.8	..	..	..	Filtered material inoculated
	232	++	2	5	++	4	3	++	4	7	+	5	3	..	..	..	..	..	100.8-102	+	3	1	
M24	241	+	3	3	..	..	..	+	6	5	..	..	..	..	..	..	..	..	99.4-100.8	..	..	..	
	242	..	..	..	..	..	..	+	7	5	..	..	..	..	..	..	..	..	99.6-103.1	+	3	1	
M25	251	..	..	..	+	7	2	+	10	3	+	5	6	+	10	1	..	..	101.8-104.7	+	7	1	
M26	261	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	101.7-105.4	+	4	1	
	262	..	..	..	..	..	..	+	10	3	+	7	1	..	..	..	..	..	102. -105.8	+	5	1	Phlegmon of abdominal wall
	263	+	2	7	..	..	..	+	11	5	..	..	..	..	..	..	..	..	101.8-107	±	2	1	Died of rabbit septicemia
M27	271	++	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	101.4-101.3	++	2	2	
M29	291	..	..	..	±	3	2	±	12	4	±	2	6	..	..	..	+	2	....	..	..	..	Died after 4 days; broncho-pneumonia
	292	±	2	2	..	..	..	+	14	5	++	3	4	..	..	..	..	..	....	..	..	..	Filtered material innoculated
R I	R I	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	..	..	..	
D I	D I	..	..	..	+*	2	..	+*	5	..	+	2	..	+	2	..	..	..	....	..	..	..	Died within 24 hours
D II	D2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	..	..	..	
Sc I	S1	..	..	..	..	..	..	+	1	..	..	..	..	..	..	..	..	..	....	..	..	..	
Sc II	S2	+	4	..	..	..	..	+	7	..	+	3	..	..	..	..	..	..	....	..	..	..	

*Scratches ?

TABLE 2

PASSAGE EXPERIMENTS AND INOCULATIONS WITH CULTIVATED VIRUS

Case	Rabbit No.	Exanthem						Desquamation			Coryza			Conjunctivitis			Enanthem		Temperature Extremes	Leukopenia			Remarks
		Erythematous			Maculopapular																		
		Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset	Duration	Intensity	Day of Onset		Intensity	Day of Onset	Duration	
M21	213	+	2	2	..	..	..	±	4	1	+	2	2	+	2	1	±?	2	102.2-102.4	+	3	1	Inoculated with filtered lung tissue from R 212
	214	±	3	1	..	..	..	..	..	..	+	3	1	..	..	..	..	..	101.6-102.2	..	..	..	Inoculated with filtered blood culture R 212
	215	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	101.6-102	..	..	..	Inoculated with nasal discharge from R 212
M27	272	..	..	..	+	5	1	+	6	2	+	5	2	..	..	..	..	..	102.3-103.6	++	7	1	Inoculated with unfiltered lung tissue R 271; died 8th day
M28	281	++	2	5	..	..	..	+	5	4	+	2	2	±	2	2	±	1	103 -103	..	..	..	Both inoculated with 2d transfer of a culture of filtered nasal washings, M 28
	282	+	2	2	+	2	2	+	7	7	±	2	2	..	..	..	+	2	102 -102.4	..	..	..	
	283	+	3	2	+	3	2	+	13	6	+	5	1	..	..	..	+	2	....	..	..	..	Inoculated with blood from R 281-282 (pooled)
	284	..	..	..	..	..	..	..	..	..	+	3	4	..	..	..	+?	3	....	..	..	..	Inoculated with blood from R 283
	285	±	3	1	+	5	5	+	10	6	++	4	6	..	..	..	..	..	....	..	..	..	Both inoculated with blood culture from R 281, second transfer
	286	+	3	4	..	..	..	+	10	5	+	3	4	..	..	..	..	..	....	..	..	..	
M29	293	..	..	..	+	6	3	+	11	3	+	2	6	..	..	..	..	..	....	..	..	..	Inoculated with culture from filtered nasal washings; patient M 29, second transfer
	294	++	2	8	..	..	..	+	9	5	++	2	4	..	..	..	++	2	102	..	..	..	
	295	..	..	..	+	4	2	..	..	..	..	..	..	..	..	..	..	..	....	..	..	..	Inoculated wtih culture from filtered nasal washings, patient M 29, third transfer
	296	+	2	1	+	3	2	..	..	..	+	7	2	+	2	1	+	2	....	..	..	..	
	297	+	4	2	+	5	4	+	10	3	+	5	2	..	..	..	..	..	....	..	..	..	Inoculated with culture from filtered nasal washings; M 29, fifth transfer

erythema, sometimes occurring without it, a maculopapular rash, which faded after 2 to 4 days and left pigmentation persisting until desquamation began.

Desquamation, either branny or flaky, occurred in all but 4 animals after the rash and was noted 3 times in cases in which no rash had been noted (3 animals with marked erythema died before desquamation occurred).

Control animals were inoculated with nasopharyngeal washings from 1 case of nasal catarrh, 2 cases of diphtheria and 2 of scarlet fever. There was desquamation in the 2 scarlet fever animals, preceded in one case by a rash which was, however, different in character from that shown by the measles rabbits. The other animal, showing 2 macules, presented other evidences of clawing and scratching over some of the unshaved portions of his skin.

Passage experiments from rabbit to rabbit were unsuccessful when nasal discharges were used, owing probably to the scantiness of material. Successful inoculations were made in 7 cases, using, 3 times, 2 cc. of blood, and, twice, a suspension of lung tissue from severe and fatal cases. The other passage experiments were conducted with rabbit blood cultures. Attempts have also been made to cultivate a specific organism from the filtered nasal washings from patients, and while the work along these lines is incomplete, the reactions obtained in rabbits inoculated with the fifth transfer of such cultures indicated at least that the virus remains alive and virulent at 37°C. for 24 days. The results obtained by reinoculating convalescent animals have thus far been rather contradictory, and frankly successful in only two cases.

It cannot be claimed that the results obtained in any one animal give a clear and typical picture of measles, yet, taking the series as a whole, there has been enough conformity to encourage the belief that rabbits are susceptible to the virus of measles and within rather wide limits give a characteristic syndrome.

NOTE.—E. Harde, in the Compt. Rend. Soc. de biol., 1921, 84, p. 968, makes a short communication on the transmission of measles from man to rabbit by means of intravenous inoculation with blood. She states that an erythema develops within 48 hours and lasts from 24 to 48 hours. The blood of a scarlet fever patient produced only a slight reddening after the same interval of time, but no real erythema. These injections were made at this laboratory a number of years ago, but the results were not considered conclusive enough to follow up or to publish, until Dr. Harde saw the rabbits described in the present articles.

THE PROPHYLACTIC USE OF MEASLES CONVALESCENT SERUM

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William H. Park and Rowland G. Freeman, Jr.

As measles is the most prevalent of the diseases of infancy and early childhood, and also predisposes to a complicating bronchial pneumonia, it is one of the two most fatal of the communicable diseases of early childhood, and its prevention has been eagerly sought by clinical and laboratory investigators. The ideal in their mind is to discover the germ and prepare a vaccine such as we have against diphtheria, tetanus and scarlet fever. Tunnicliff in the United States and Caronia in Italy have reported such discoveries. Their work is now being subjected to crucial tests.

TABLE 1.

TOTAL DEATHS AND DEATHS FROM MEASLES

	1918	1919	1920	1921	1922
Total deaths	98,119	74,433	73,249	64,257	69,690
Total deaths under 5	21,019	15,744	17,288	13,672	14,847
Total deaths from measles	790	218	736	165	977
Deaths from measles under 5	734	210	687	150	917
Percentage under 5	4	1	3.5	1	6

Some thirty years ago, a German physician used large amounts of serum from convalescents in the treatment of several cases in which the symptoms were just beginning. The results were favorable, and he suggested that it might be used as a preventive measure.

Since 1921 the convalescent serum has been utilized with excellent results in the hospitals for contagious diseases in New York City, in children who have been exposed by the development of measles in the wards. A grant of money by the Metropolitan Life Insurance Company made it possible to attempt to provide enough serum for all exposed inmates in children's institutions in New York City, and also for all the children in the city who were under three years of age.

Results Obtained in New York City

More than 1,500 children received preventive injections between December 1 and June 1. From 979 of these we have fairly accurate data as to the date and degree of exposure, the amount of plasma injected and the success attained.

Of these 979 children, 753 were exposed in institutions and 226 in private families. In 404 of the institutional children and in all of the children in families, the exposure was direct. In 349 it was indirect, in that actual personal contact with a measles patient was not proved.

No bad effect followed the injections in any case. Only one of the children died, and this one had an empyema before contracting modified measles and was only 2 years of age. We do not think that the attack of measles appreciably hastened its death.

TABLE 2.

DATA IN INSTITUTIONAL CHILDREN RECEIVING PREVENTIVE INJECTIONS

Amount of Plasma Injected	Number of Children	Modified Measles, Per Cent.	Unmodified Measles, Per Cent.	Complete Success, Per Cent.	Incomplete Success, Per Cent.
3 cc.	219	20	8	72	92
4 and 5 cc.	190	8	2	90	98
6 to 10 cc.	243	6	2	92	98
Total in institutions.....	753	11	5	84	95
Total in families........	226	42	5	52	95

CONCLUSIONS

The injection of 6 cc. of convalescent serum or plasma into a child under 3 years of age and from 6 to 10 cc. for a child over that age who has been exposed for less than five days to measles, is sufficient, as a rule, to prevent infection. If it does not, it almost certainly so modifies the attack that it is very mild and is not likely to lead to complications.

The prompt use of the serum will prevent serious outbreaks of measles from developing in institutions and so prevent the development of complicating pneumonias.

It would be of extreme value to have a supply on hand to prevent outbreaks of measles among troops, such as occurred so disastrously in the late war.

The serum gives immunity for only two weeks to one month. In the modified cases there is lasting immunity.

The serum gives the greatest accumulation of antibody shortly after the convalescence of the patient. At the end of three months, the antibodies are still abundant but probably less in amount.

In order that the use of convalescent measles plasma may become general, some plan must be devised by which the supply will be constant. It would seem that if every physician equipped himself with the very simple apparatus necessary for obtaining the blood, and if the attempt was made to draw between 10 and 25 cc. of blood from every case seen in children, and larger amounts in adults, he would have enough for the majority of exposures in his practice, the average dose being only 5 cc. Institutional needs must be met by other methods, such as the one to which the New York City Department of Health had recourse. The Department of Health has a standing offer to send a physician to any convalescent adult who has given consent to have his blood drawn. After the blood is withdrawn a fair proportion is kept for the use of the physician who referred the case to the department.

THE RELATION OF THE TUNNICLIFF AND FERRY DIPLOCOCCI TO MEASLES

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William H. Park, Anna W. Williams and Minnie Wilson

This paper is entirely concerned with the study of the diplococcus which has been suggested as the cause of measles by Dr. Tunnicliff and of similar green producing streptococci claimed to be the cause of measles by Dr. Ferry.

As is well known, Tunnicliff in 1917 described a micrococcus which she obtained from the blood of early cases of measles. She did not obtain this regularly from the cases at first, giving as her reason that they grew with difficulty, but later she almost never failed to obtain them. There were certain cultural differences in the strains. For instance, one strain was definitely an anaerobe and some did and some did not produce acidity in milk. She believes that with this culture she successfully produced measles in rabbits and noted that these rabbits after their recovery became resistant to later inoculations. These effects were produced not only with the primary cultures but with those of the fifth transfer. She observed that strains of other types of streptococci obtained from the blood either early or late in the disease did not produce measles. This seemed to her to make unlikely the claim that it was not the diplococci but rather a filterable virus which had grown in symbiosis with the supposedly specific green diplococci, which produced the disease. She found that while a filtrate alone or the dead diplococci alone failed to give any distinctive diagnostic cutaneous reaction, that the whole culture fluid containing both cultures and toxic fluid did give differential results between immune and non-immune children and animals. The serum of immunized animals also neutralized the toxic culture fluid. She stated that the mixed culture fluid and organisms regularly produced a reaction in the skin of children who had not had measles, but not in convalescent measles patients or in 96 per cent. of those who gave a history of having had measles. She further stated that the serum of goats injected with the cultures, when injected into children within two days of exposure to measles, prevented the disease in the majority and modified it in the others a little later.

Ferry obtained from the blood of an early case of measles a green streptococcus which made a strong toxin. In his hands 0.1 cc. of a 1 to 400 dilution of the filtered toxin produced a skin reaction in the great majority of susceptible children and very rarely in those who had had measles. He produced in animals an antitoxin for the toxin. He considered his culture as distinct from the ones isolated by Tunnicliff, because of her statement that hers was a diplococcus and that it did not make a soluble toxin. In our hands both cultures make a toxin, but that from Ferry's culture is much stronger. They seem to us to be similar organisms.

The published reports of Tunnicliff and of Ferry certainly seem to present very impressive evidence of the etiological significance of a diplococcus growing in short chains. There were, however, several difficulties which prevented us from being completely convinced. It seemed difficult to understand how a diplococcus which appeared in enormous numbers in

TABLE 1.

SHOWING PERCENTAGE OF CASES FROM WHICH GREEN STREPTOCOCCI (NON-HEMOLYTIC) WERE ISOLATED AT VARIOUS STAGES OF MEASLES

			Green Streptococci		Negative
			Positive	Doubtful	
Stage of Disease	No. of Cases	Percentage of Cases from which Green Colonies Were Isolated	Green and Ascitic Colonies Like Tun. No. of Case	Green Col. Like Tun. Ascitic Col. Not Like Tun. No. of Cases	Col. Not Green Morphology of Col. Like Tun. Ascitic Col. Not Like Tun. No. of Cases
Pre-eruptive	20	70	13	2	5
First day rash	19	89	16	1	2
Second day rash	11	72	7	1	3
Old rash	7	71	5	0	2
Controls:					
So-called influenza	9			9	

The cultures in our "Positive" column correspond to the description given by Tunnicliff of the colonies isolated from rubeola. The following is her description: On blood agar the colonies are small punctate, green. On ascitic agar, the colonies are small, round, homogeneous, smooth-edged.

the discharges from the eyes, nose and throat during the catarrhal stage of measles should be absent in cases, as shown by its lack of contagiousness, from the upper respiratory tract during the period of incubation. If measles were due to this diplococcus it would seem probable that, like diphtheria and scarlet fever, soon after the transfer of the infecting microbe it would begin to develop and produce symptoms and spread infection.

Again in almost all diseases due to bacterial infection there are a greater or lesser number of cases in which the infecting microbes persist. Cultures as well as transmitted infection to others reveal this. Measles, like smallpox, mumps, chickenpox, yellow fever and most other diseases supposed to be due to filterable viruses, has a long period of incubation when it is not communicable and then, when convalescence is established, it suddenly ceases to spread contagion.

While impressed by the evidence submitted by Tunnicliff and Ferry we were still doubtful and we felt it wise to undertake seriously an attempt to corroborate their findings. In order to make these studies possible we applied for and obtained a grant of money from the Milbank Memorial Fund. To our force we added a number of technical assistants.

Our first endeavor was to discover through cultures whether the diplococci were regularly present in the pre-eruptive and eruptive stages and whether the strains were the same in the different cases. It is only fair to state that the cultures tested by us were from the mucous membranes and not from the blood, but many of them were identical with either the Tunnicliff strain or the Ferry strain. The results of our work are shown in the tables presented here.

Table I gives the results so far as the appearance of colonies on blood and ascitic agar indicate identity.

The results viewed from the etiological standpoint are perplexing. Eighty-four per cent. of the cultures from the measles cases gave characteristic colonies on both blood and ascitic agar, while in cultures from 9 control cases characteristic colonies were not found. They were absent, however, in the cultures from 16 per cent. of the measles cases and their persistence during the period of the fading rash was somewhat disquieting since the cases had reached a stage when some were supposed to be but slightly and others not at all communicable.

Twenty-two of these strains (see Table 2), together with the Tunnicliff and Ferry strains sent us by these investigators were studied as to their action on carbohydrates. Six different groups were found, only two of which contained many cultures, namely, the Tunnicliff and our 67 T. It is striking that the Ferry and Tunnicliff strains are different as to their action on salicin and inulin. These findings are somewhat disconcerting and rather suggested the possibility that these diplococci are complicating micro-organisms. We then tested different strains for their reaction to the agglutinin developed by the Tunnicliff strain, the Ferry strain and our 67 T strain. (See Table 3.)

TABLE 2.

22 STRAINS TYPED BY CARBOHYDRATES AS COMPARED WITH TUNNICLIFF'S AND FERRY'S

Type	Maltose	Salicin	Inulin	Mannite	Raffinose	No. of Our Strains in This Type: No.	Stage of Disease
Tunnicliff	+	+	+	—	+	6	2 pre-eruptive 3 1st day rash 1 8th day convalescence
Our 2. E..........	+	—	+	—	—	2	1 pre-eruptive. This is tonsil type of culture case 1 3rd day of rash
5. T..........	+	—	+	—	+	2	4th day of convalescence
4. T..........	+	+	+	—	—	0	
67. T..........	+	+	—	—	+	7	1 pre-eruptive 1 1st day of rash 5 2nd day of rash
Ferry	+	—	—	—	+	1	11th day of convalescence

The strains shown in Table II are similar to Tunnicliff's on blood-agar, ascitic-agar and in bile test.

TABLE 3.

COMPARISON BY DIRECT AGGLUTINATION OF OUR GREEN STREPTOCOCCI WITH TUNNICLIFF'S AND FERRY'S

Cultures Isolated from Various Stages of Measles

			Agglutinin Serums					
			Tunnicliff		Ferry 01860		Our 67-F	
Stage of Disease	Bacterial Suspensions Strain Number	Rabbit Serum No.	Titre with Homologous Antigen	Titre with Other Strains	Titre with Homologous Antigen	Titre with Other Strains	Titre with Homologous Antigen	Titre with Other Strains
	Tunnicliff ...	20	3200		1600	—	3200	3200
	Ferry 1		3200	—	1600		3200	—
	Ferry 2		3200	—	1600	1600	3200	—
Pre-eruptive	67-T.........	31	3200	3200	1600	—	3200	
	2-E.........	20	3200	—	1600	—		
	T.........		3200	—	1600	—		
	4-T.........	C	800	—	1600	1600		
	9-E.........		800	—	1600	—		
	27-E.........	20	3200	100	1600	—	3200	—
	33-T.........		3200	—	1600	—	3200	—
	91-E.........	20	3200	—	1600	—	3200	—
First Day Rash	1-E.........	20	3200	—	1600	—	3200	—
	N.........		3200	—	1600	—	3200	—
	18-E.........	C	800	—	1600	1600		

— Means no agglutination in 1:100 dilution.

Explanation of Strain Number: The numeral refers to serial number of the case, the letter to source of the culture: E (eye), N (nose), T (tonsil)

It is noted that the rabbit blood serum which had been removed from the rabbit treated by injections of the Tunnicliff strain agglutinated strongly only one of the strains other than the Tunnicliff and only one other in very low dilution. The serum from the Ferry rabbit serum agglutinated 2 of the 12 strains, and the serum from our 67 T none of the 7 strains tested. We then tested the strains by agglutinin absorption and found the same results as by direct agglutination. (See Table 4.)

TABLE 4.

OUR GREEN STREPTOCOCCI FROM MEASLES COMPARED WITH TUNNICLIFF'S AND FERRY'S BY AGGLUTININ ABSORPTION

		Agglutinin Serums				
		Tunnicliff			Ferry 01860	
Stage of Disease	Absorbing Cells Strain No.	Rabbit Serum No.	Titre with Homologous Antigen Before Absorption with Cells Listed Under "Strain Number"	Titre with Homologous Antigen After Absorption	Titre with Homologous Antigen Before Absorption	Titre with Homologous Antigen After Absorption
	Tunnicliff	20	3200	—	1600	1600
	Ferry 1..		3200	3200	1600	—
	Ferry 2..		3200	3200	1600	—
Pre-eruptive	67-T.....	20	3200	—	1600	800
	2-E.....		3200	3200	1600	1600
	T.....		3200	3200	1600	400
	4-T.....	C	800	800	1600	1600
	9-E.....		800	800	1600	800
	27-E.....	20	3200	1600	1600	800
	33-T.....		3200	3200	1600	400
	91-E.....		3200	3200	1600	800
First Day Rash	1-E.....		3200	3200	1600	1600
	N.....		3200	3200	1600	1600
	18-E.....	C	800	800	1600	200

— Means no agglutination in 1:100 dilution.

Explanation of Strain Number: The numeral refers to serial number of the case, the letteer to source of the culture: E (eye), N (nose), T (tonsil).

These results are not incompatible with the possibility that, while these strains differ so markedly in their reaction to specific agglutinins, they may produce the same toxin. We know that there are many different agglutinative types of diphtheria bacilli, scarlet fever streptococci and pneumococci, but the great diversity of these strains suggests that they, like the influenza bacilli which have the same great diversity, may be only complicating slightly pathogenic bacteria.

When the work was begun we planned to make cultures from a few of the cases from time to time from the beginning of the disease until after convalescence, but in the mass of work the making of the later cultures was put off until June, when the cases suddenly ceased. We had intended to test the early and later cultures from the same case for their identity in relation to specific agglutinins. This work is now being undertaken but it was impossible to complete any of the cases in time to make a report at this time.[1] We can state, however, that in 3 cases of measles the green diplococci persisted up to the tenth, twenty-first and twenty-third day after the appearance of the rash and that, so far as cultural appearance could determine, the diplococci which appeared in the first cultures made early in the disease persisted to the final cultures made during convalescence and after. (See Table 5).

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[1] October 13, 1926.

TABLE 5.

SHOWING PERCENTAGE OF GREEN STREPTOCOCCI IN CULTURES FROM CONVALESCENT MEASLES

Case Number	Days Convalescent	Eye Amount Growth	Eye Per cent. Green	Nose Amount Growth	Nose Per cent. Green	Tonsil Amount Growth	Tonsil Per cent. Green
98	6	+	100	+++	0.1	+	100
	8	+	100	+	100	No. growth	
	21	+	90	+	75	+++	95
99	11	++	95	++	90	++	99
	16	No growth		±	95	±	99
	23	+	45	+	25	++	50
100	6					+	50
	14					++	50
101	3					±	100
	7					+	50
	10					±	100

± = Scant growth. + = Moderate growth. ++ = Abundant. +++ = Profuse.

If further investigation proves them to be identical it would require conclusive proof to make us accept them as the cause of measles, for it is well established that cases of measles are not communicable at such a period after convalescence and we have no knowledge of such a rapid loss of virulence in streptococci in convalescence.

Drs. Tunnicliff and Ferry both claim that, when the toxin and dead organisms are used in proper dilution in intracutaneous tests, they are diagnostic between immunity and lack of immunity to measles.

We have observed the results of tests made in hundreds of young children, some of whom have had measules and some of whom have not,

TABLE 6.

TESTS MADE WITH TUNNICLIFF MEASLES TOXIN, WILLARD PARKER

Case	Age	Toxin 1:20 3	Dilutions 1:40 3	Toxin 1:10 aa Convalescent Serum 1:2	Broth Control 1:20
	"Suscept bles"—*i.e.*, those who never had measles				
1	4 years	1 x 1 P	1 x 7 PP	.8 x .8 P	
2	16 months	.4 x .4 P	.4 .4 P	1.5 x 1.5 P	.5 x .4 P
3	2 years	.4 x .3 P	.3 x .3 P	.5 x .5 P	.2 x .2 P
4	15 months	1.5 x 1.5 P	1 x 1 P	.6 x .* P	.5 x .5 P
5	14 months	.5 x .4 P	.7 x .6 P	.5 x .6 P	.3 x .4 P
6	4 years	1.5 x 1.5 P	2 x 2 P	.5 x .5 P	
7	6 years	.5 x .6 PP	.3 x .3 PP	.9 x .9 P	
	"Non-susceptibles"—*i.e.*, the following children give a definite measles history				
1	3 years	1.2 x 1.1 P	.9 x 1.1 P	2.5 x 2.5 BP	
3	2½ years	.7 x .8 P	.5 x .* P	.6	
2	2½ years	1.2 x 1.3 P	.7 x .8 VPP	1.5 x 1.7 PP	.5 x .4 P
4	2 years	.5 x .5 P	.4 x .4 P		.2 P
5	1 year	1.2 x 1.2 P	1.5 x 1.6 P	1.5 x 1.4 P	
6	3 years	.2 x .2 PP	.2 x .2 PP	.6 x .7 P	
7	4 years	1.5 x 1.5 P	.4 x .5 PP	1 x 1.2 BP	

The tests were read at the end of 18 hours.
P = Pink. BP = Bright Pink. PP = Pale Pink. VPP = Very Pale Pink.
1.2 x 1.5 = 1.2 centimeters by 1.5 centimeters.

and we have been unable to obtain distinctive results. Dr. Schroder and Dr. Zingher have obtained very similar results in the children tested by them.

Three years ago with Freeman and Valentine we tried to see if it were possible to get in some medium the development of some toxin in cultures from filtered nasal secretion of early cases of measles. In only one case did we get a toxin, and this curiously enough was in a culture in which a diplococcus like the Tunnicliff diplococcus had passed the filter. This gave us fairly distinctive differences between susceptible children and those who had recently recovered from measles. Those who had not had it recently reacted quite irregularly.

It seemed to us, therefore, that there might have been some reaction to the toxin of the diplococci in the blood and in the respiratory passages but that this in no way proved that they were the original etiological factor. Table 6 gives a characteristic test. It is seen how irregular the reactions were. Certainly they would not suffice to test the immunity of the children. There was practically no difference between the two groups. Similar results were obtained with the Ferry toxin.

EFFECT OF INTRACUTANEOUS INJECTIONS OF THE SERUM OF IMMUNIZED GOATS

Tunnicliff describes the result of injecting her diluted immunized goat serum intracutaneously into children before the measles rash has developed and notes that, just as has been shown to occur with convalescent measles serum, the injection of serum from goats which have received injections of her diplococcus antigen inhibits the development of the rash. Long before her paper appeared we had tried her goat serum, the Ferry serum and serum from goats immunized with our strains intracutaneously in children. Our strains were from cases in the Koplik spot period. The results with all these serums were striking and for a time we felt that we had strong evidence as to the specificity of the diplococci. In scarlet fever, in which intracutaneous injections of convalescent human serum or of antitoxin developed in the horse blanches the developed rash, we think the effect is due to a direct antitoxic action. In preventing the development of the measles rash we are less certain that this is a specific antitoxic reaction. Having obtained these striking results, we tried as controls the serum of a goat which had been immunized to scarlatinal streptococci and one which had received

TABLE 7.

MEASLES EXTINCTION TESTS

Number of Case	Human Measles Convalescent Serum	Goat Serum Our Strains	Goat Serum Tunnicliff Strain	Goat Serum Ferry Strains	Control Animal Serum	Control Animal Serum
	1:10	1:10	1:10	1:10	1:10	1:10
5	2. x 2. F	2. x 2. M			3. x 3.5	1.5 x 1.5 F
6	4. x 5.	4. x 3.			3. x 4.	
9	4. x 5.	3. x 3.	2. x 3.	4. x 3.		
4	3. x 3. M	3. x 3. M	3. x 3.			
9	4. x 5. M	3. x 3.	2. x 3.	4. x 3.		
12	4. x4.	3. x 3.		1. x 1.		

F = Faint blanching. M = Marked blanching.

diphtheria bacilli and one which had received, so far as known, no injections. The results were surprising. The serum from one of the control goats, when given intracutaneously, inhibited the rash almost as well as did the convalescent measles serum or the Tunnicliff serum and two of the others had definite but not so decided action. Some of these tests are shown in Table 7.

These surprising results throw doubt on the value of the measles rash extinction tests as indicating a specific neutralization of toxin.

We close our paper at this point since we have had no experience with cultures reported upon by Caronia or Degkwitz. The results obtained by Degkwitz have impressed us very strongly and we hope to try and corroborate his findings in the near future.

For bibliography see the original article.

EXPERIMENTAL MEASLES IN RABBITS AND MONKEYS

Published in The Journal of Infectious Diseases, 1921, Vol. XXIX, pp. 429-436

Mary Nevin and Florence R. Bittman

In our search of the literature on experimental measles we have been unable to find any mention of attempts having been made to transmit the virus to rabbits. Anderson and Goldberger speak of the apparent insusceptibility of animals other than the monkey to the inoculation of measles virus, but no reference is given.

In order to test the receptivity of rabbits to the infection of measles, the blood of 6 patients with measles was inoculated into 6 rabbits. Three of the rabbits were inoculated with blood drawn on the second day, 2 rabbits with blood drawn on the third and 1 with blood drawn on the fourth day after the onset of the disease. In all we inoculated 17 rabbits, 6 with human blood and 11 with the blood of rabbits that had given evidence of a reaction. The inoculations were given intravenously in amounts varying from 1 to 15 cc. All of the rabbits inoculated with human blood gave evidence of a reaction. So also did all but of the 2 rabbits subinoculated from these 6 rabbits. Passage inoculations for subsequent injections into monkeys were carried out only from patient "S."

Symptoms in Rabbits

Fifteen rabbits developed symptoms in from 3 to 7 days. The symptoms were not so marked as in monkeys. In some instances there was a rise in temperature coincident with a decrease in the total leukocyte count, but this was by no means constant. We noted also that in the majority of the rabbits there was frequently a leukocytosis on the second, third or fourth day after injection followed by a decrease in the total leukocyte count. The fact that these findings were not constant, together with the fact that we had found great fluctuations in the daily counts of these animals for one week prior to their inoculation, led us to disregard these changes in the white blood counts.

Of the 15 rabbits showing symptoms of measles, 10 developed small hyperemic, slightly elevated spots on the labial mucosa; in 5 instances these spots showed whitish centers. Twelve rabbits developed, in from 2 to 4 days, a marked conjunctivitis with a moderate edema of the lids and lacrimation. Four rabbits developed a slight diarrhea.

In from 3 to 8 days all of the 15 animals showed erythema of varying degrees, from a slight flush over the chest and in the axillary region to a general redness extending to the groin. A redness of the skin always followed the shaving of the rabbits, but, unlike the control animals noted in the following, the flush became more intense in from 3 to 8 days.

In no instance was a distinctly typical exanthem noted. In 8 rabbits we noted several erythematous macules on the chest and abdomen. Such a rash was not distinctive. Neither is the reaction on the skin of rabbits after inoculation with cowpox vaccine the typical one that takes place in human beings.

In all of the 15 rabbits a marked desquamation occurred, beginning from the fifth to the fourteenth day. Both the shaved and unshaved portions of the neck, chest, axillae, abdomen and groin were involved. In two instances the desquamation was fine and branny; with the remaining 13 animals large flake-like scaling took place.

From one of the human cases, "S," the virus was passed through 5 rabbits (Charts 1, 2 and 3). On the sixth day after inoculation, the fifth rabbit was bled from the heart and the citrated blood injected intravenously into monkey 8, M. rhesus. The monkey developed a leukopenia in 3 days (Chart 4A). On the fourth day there was loss of appetite, slight reddening of the eyelids and lacrimation with a marked erythema over the face, neck and shoulders, while on the labial mucosa two discrete, elevated, hyperemic macules with bluish white centers appeared. On the fifth day a maculopapular rash appeared about the mouth, cheeks and forehead. On the sixth day, over the bluish abdomen of the monkey, small clusters of yellowish, elevated papules appeared. From the sixth to the ninth day there was a moderate erythematous, granular rash on the mucous membrane of the lips. The papules on the labial mucosa disappeared on the eighth day. The rash also began to fade and marked desquamation was noted on the face, chest, shoulders, abdomen and in the groin.

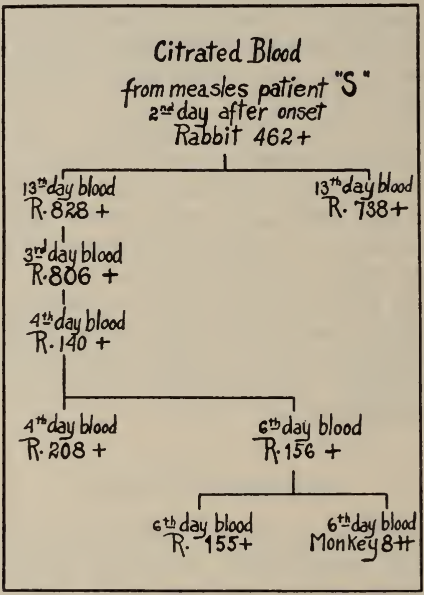
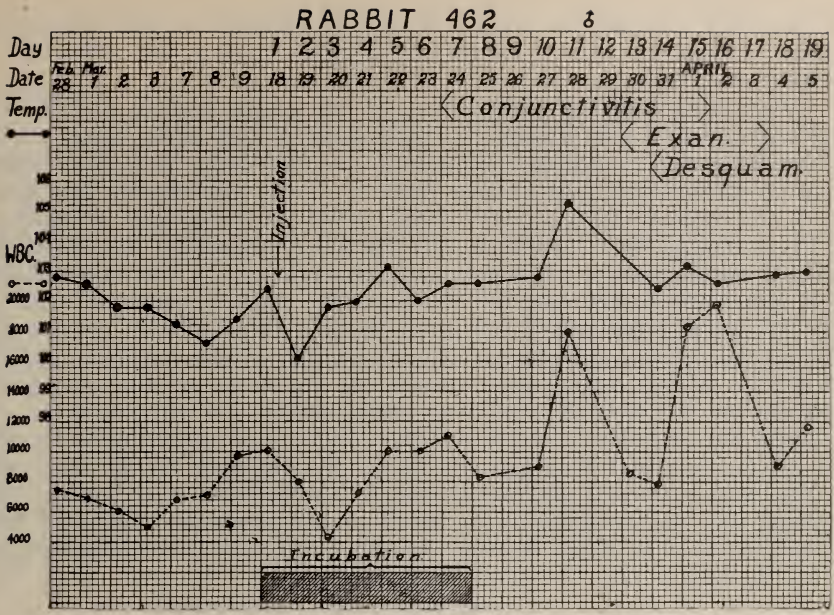


Chart 1.—Transmission of measles virus from case "S" through 5 rabbits to a monkey; suggestive reaction +, typical measles reaction ++.

(Reprinted by courtesy of The Journal of Infectious Diseases.)

Blake and Trask have shown that monkeys inoculated intratracheally with nasopharyngeal washings from patients with measles develop characteristic symptoms of measles. Accordingly, we reinoculated monkey 8, M. rhesus, one month later with nasopharyngeal washings containing another strains of measles virus (case "O"). The monkey failed to react (Chart 4B). The control (monkey 24, M. rhesus, chart 5A) developed the characteristic symptoms of measles in 6 days.



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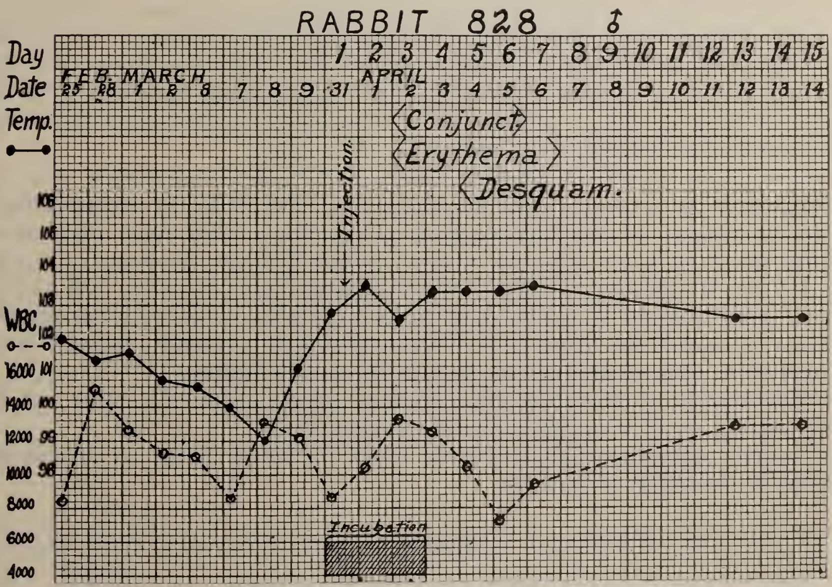


B

Chart 2.—Transmission of measles virus, strain from case "S," by means of intravenous inoculation of whole citrated blood; A, rabbit 462 from case "S"; B, rabbit 828 from rabbit 462.

(Reprinted by courtesy of The Journal of Infectious Diseases.)

In order to test the receptivity of the monkey to the infection of measles with blood from a human being with measles the following experiment was made:

Two patients with measles, "Y" and "O," were bled on the third day after the onset of the disease. In both cases Koplik spots were present. Conjunctivitis and coryza were present in one case only. Both cases showed a maculopapular rash. The citrated blood of these two patients was pooled and 10 cc. was inoculated intravenously within an hour into monkey 26, M. rhesus (chart 5B). On the fourth day after inoculation the animal was drowsy; loss of appetite was noted, and the diminution in the total leukocyte count was marked. The monkey developed no distinct symptoms until the sixth day when a few bright red spots, slightly elevated, were noted on the labial mucosa. On the seventh day discrete, slightly raised, hyperemic macules with bluish white centers were present on the inside of the cheeks; the enanthem on the labial mucosa was more marked but not coalescent. On the eighth day we noted a few flat reddish macules, disappearing under pressure, on the face and shoulders. Conjunctivitis appeared on the ninth day; the lids were thickened and hyperemia and lacrimation were present. The following day the exanthem had spread to the chest and abdomen, while the rash on the face of the monkey showed a yellowish brown pigmentation. During the next 4 days the exanthem gradually faded, the spots on the chest and abdomen becoming a yellowish brown also. Desquamation started on the eighth day, but was never marked. By the fourteenth day the animal appeared well; the exanthem had entirely faded and only a slight pigmentation and desquamation were noted.

Controls.—Eleven rabbits were shaved over the neck, chest, axillae, abdomen and groin. Six rabbits were inoculated intravenously each with 5 cc. of citrated blood as follows: two with blood from two cases of diphtheria, one from a case of lobar pneumonia, one from a case of lethargic encephalitis, one with normal human blood and one with normal rabbit blood. The total leukocyte count of all these rabbits showed considerable variation from day to day with the exception of the rabbit inoculated with normal rabbit blood. This count remained fairly constant.

The 5 uninoculated rabbits were kept under observation for the appearance of an erythema and desquamation. All of the 11 control animals showed a redness of the skin incident to shaving. This flush disappeared in from 48 to 72 hours whereas, in the animals showing evidence of a reaction to the inoculation of blood from patients with measles, the redness became more marked from the third to the eighth day following the inoculation and persisted for from 3 to 5 days and then gradually faded. In no instance was desquamation noted in the 11 control rabbits.

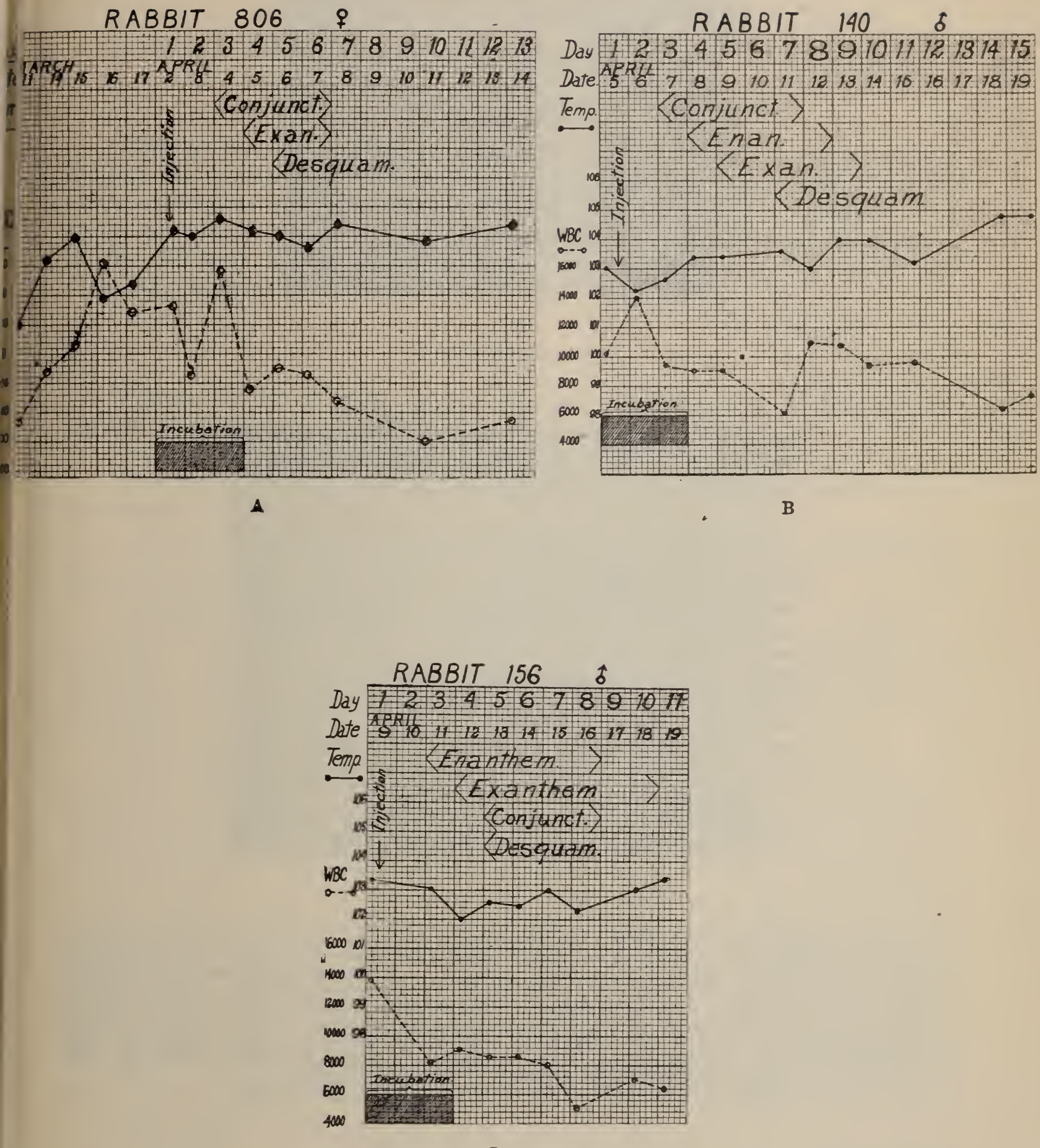
Microscopic examination of scrapings made from the spots on the labial mucosa showed no parasitic invasion.

Aerobic and anaerobic cultures made of blood prior to inoculation showed no evidence of growth.

SUMMARY

Blood from 6 patients with measles was inoculated into rabbits. The animals gave evidence of infection.

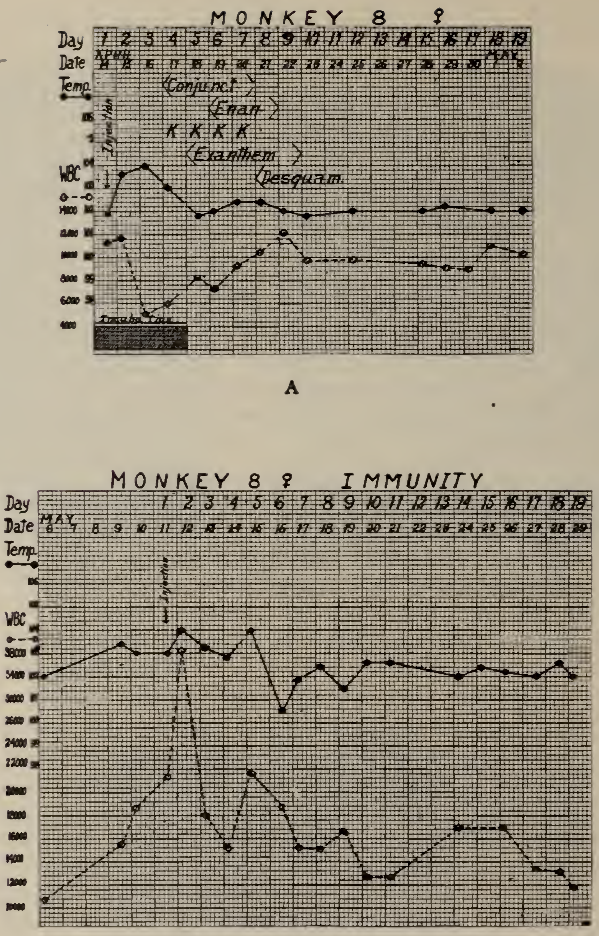
Passage from one human case of measles was carried on through 5 rabbits, and a monkey inoculated with the blood of the fifth rabbit gave typical symptoms of measles.



A B C

Chart 3.—Transmission of measles virus strain from case "S" by means of intravenous inoculation of whole citrated blood; A, rabbit 806 from rabbit 828; B, rabbit 140 from rabbit 806; C, rabbit 156 from rabbit 140.

(Reprinted by courtesy of The Journal of Infectious Diseases.)



A

B

Chart 4.—Observations on monkey 8; A, transmission of measles virus strain from case "S" by means of intravenous inoculation of whole citrated blood from rabbit 156 (chart 3C); B, attempted reinfection by means of intratracheal inoculation with 10 cc. of nasopharyngeal washings containing another strain of measles virus (case "O"). The rise in the total leukocyte count prior to inoculation was due, in all probability, to a slight diarrhea. This condition rapidly ceased on change of food. As Blake and Trask have noted, the sharp rise following the injection of nasopharyngeal washings is probably due to the effect of other organisms. This animal showed no reaction to the virus and was discharged on the nineteenth day. Control monkey 24 (chart 5A), inoculated with 10 cc. of the same washings developed the characteristic symptoms of measles in 6 days.

(Reprinted by courtesy of The Journal of Infectious Diseases.)

A monkey inoculated with pooled blood from two human cases of measles taken on the third day after the onset of the disease gave the characteristic symptoms of measles.

Blood from cases other than measles, when inoculated into rabbits, failed to give evidence of infection.

For bibliography see the original article.

FURTHER NOTES ON EXPERIMENTAL MEASLES IN RABBITS AND MONKEYS

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Mary Nevin and Florence R. Bittman

During the past winter confirmation of our work on experimental measles in rabbits and monkeys[1] was undertaken. Blood from 3 early cases of measles was used for the inoculation of 2 series of animals. The blood was drawn on the second day of the disease from patients with measles in the Willard Parker Hospital for Contagious Diseases and within an hour was injected into the animals.

Case Histories

Patient Z: Ill 2 days, coryza, purulent conjunctivitis, Koplick spots, general maculopapular rash.

Patient Q: Same symptoms as patient K.

Patient Z: Ill 2 days, coryza, purulent conjunctivitis, Koplick spots, general maculopapular rash.

Series 1.—The bloods of patients K and Q were drawn on the same day, pooled and inoculated into 1 monkey and 3 rabbits (chart 1). The inoculations were intravenous, and citrated blood was used.

Monkey 31 (M. rheus) received 12 cc. of blood and developed a leukopenia on the fourth day; this was followed on the next day by an enanthem. Then Koplik spots developed and on the sixth day a general maculopapular rash was noted. A fine branny desquamation began on the ninth day.

Three rabbits (1422, 1423, 1425) inoculated with 3.5, 4 and 5 cc. respectively, developed reactions on the fourth and fifth days. The reaction consisted of lacrimation, erythema and exanthem followed by desquamation. One of these rabbits (1422) was bled on the eighth day after inoculation and 3 rabbits (1432, 1433, 1438) were inoculated with 4, 6 and 7 cc. respectively, of the citrated blood. On the second day rabbit 1438 developed an enanthem which lasted for 4 days. The entire labial mucosa was hyperemic, the lips thickened and swollen and the lower labial mucosa intensely injected. No Koplik spots were noted. The exanthem appeared on the eighth day and was followed by a flaky desquamation. No enanthem was noted in the other 2 rabbits, but the exanthem which appeared on the fourth and seventh days was marked. A general maculopapular rash was present on the chest, axillae, and groin; brownish pigmentation and large flaky desquamation followed on the eleventh day.

Two rabbits (1444, 1448) were inoculated with 6 and 7 cc., respectively, of citrated blood drawn from rabbit 1438 on the eighth day. Both of these animals developed reactions, and rabbit 1444 was bled on the fifth day.

Rabbit 1454 inoculated in turn with 10 cc. of this blood showed a marked erythematous rash on the third day. From this animal, 10 cc. of blood was drawn on the fourth day and inoculated into monkey 86 (M. rhesus). A leukopenia occurred on the fourth day. On the following day an enanthem was noted on the upper and lower labial mucosa. No other symptoms were noted until the eighth day when a marked exanthem appeared on the face, neck and shoulders; the rash spread to the abdomen and groin, and gradually faded; desquamation commenced on the tenth day.

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[1] Jour. Infect. Dis., 1921, 29, 4, p. 429.

As seen in chart 1, these typical symptoms in the monkey developed after four rabbit passages.

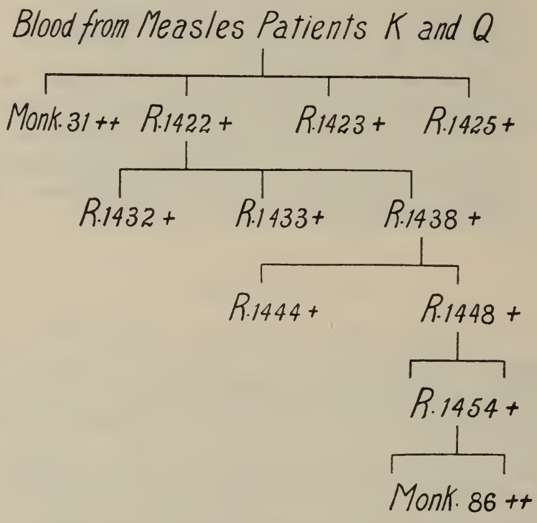

Chart 1.—Transmission of measles virus from measles patients K and Q through four rabbits to a monkey. Suggestive reaction +; typical reaction ++.

(Reprinted by courtesy of The Journal of Infectious Diseases.)

Series 2.—Measles virus from the blood of patient Z (chart 2) was successfully carried through 3 rabbits and 3 monkeys (M. rhesus) without any apparent diminution in its ability to produce typical measles symptoms in the monkey. Our idea in passing the virus from monkey to monkey at the end of the experiment was to eliminate as far as possible all question of a serum rash caused by the injection of blood from a different species of animal.

Citrated blood in amounts varying from 2.5 to 10 cc. was used throughout, with the exception of the last passage to monkey 3 when defibrinated blood was used. All inoculations were intravenous. The symptoms are recorded in the chart.

In order to note the appearance of the rash, all of the rabbits in both series were shaved the day before injection. Our previous experience had shown that careful shaving of rabbits produces no lasting irritation which would be confused with a true erythematous rash. White blood counts were made on all animals. In the case of the rabbits, the results confirmed our previous findings that the white blood count of these animals is inconstant and cannot be relied on as a diagnostic point in rabbits.

Controls.—In our previous work we had used 11 rabbits as controls, giving them intravenous injections of blood from diseases other than measles (diphtheria, lobar pneumonia, lethargic encephalitis), also normal human blood and normal rabbit blood. As none of these rabbits showed any reaction to these injections, we did not deem it necessary to repeat this part of the work.

A normal monkey was, however, injected intravenously with 5 cc. of normal defibrinated human blood and gave no reactions to the injection. Daily blood counts on this animal showed no leukopenia nor did an enanthem or exanthem develop.

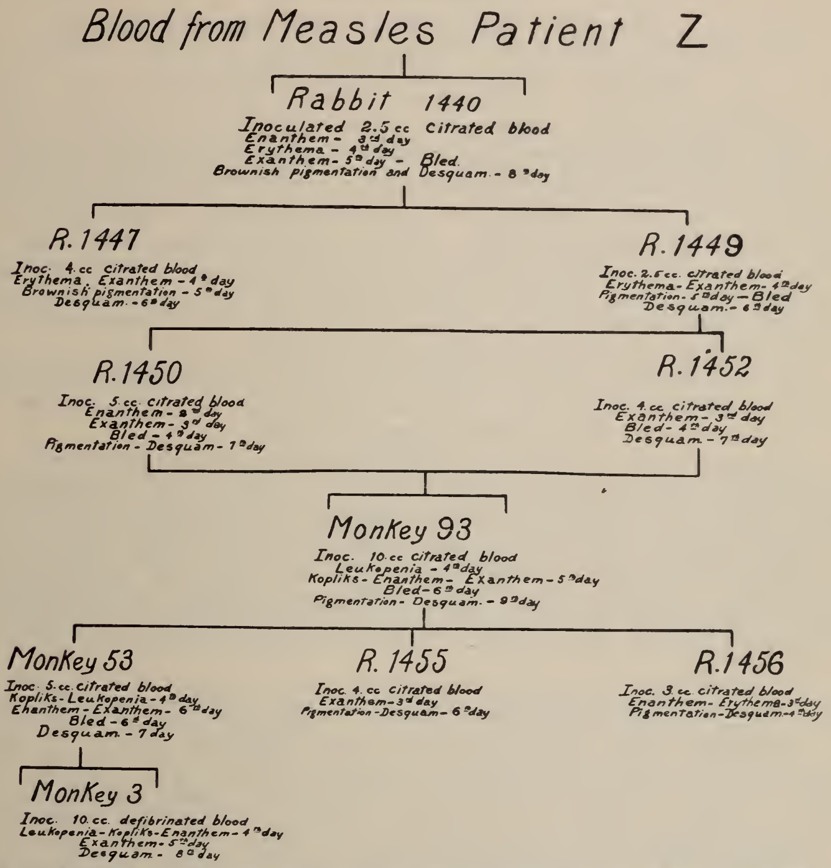
Blood from Measles Patient Z
Rabbit 1440
Inoculated 2.5 cc Citrated blood
Enanthem - 3rd day
Erythema - 4th day
Exanthem - 5th day - Bled.
Brownish pigmentation and Desquam. - 8th day
R. 1447
Inoc. 4. cc citrated blood
Erythema. Exanthem - 4th day
Brownish pigmentation - 5th day
Desquam. - 6th day
R. 1449
Inoc. 2.5 cc. citrated blood
Erythema - Exanthem - 4th day
Pigmentation - 5th day — Bled
Desquam. - 6th day
R. 1450
Inoc. 5. cc. citrated blood
Enanthem - 2nd day
Exanthem - 3rd day
Bled - 4th day
Pigmentation - Desquam - 7th day
R. 1452
Inoc. 4. cc. citrated blood
Exanthem - 3rd day
Bled - 4th day
Desquam. - 7th day
Monkey 93
Inoc. 10. cc citrated blood
Leukopenia - 4th day
Kopliks - Enanthem - Exanthem - 5th day
Bled - 6th day
Pigmentation - Desquam. - 9th day
Monkey 53
Inoc. 5. cc. citrated blood
Kopliks - Leukopenia - 4th day
Enanthem - Exanthem - 6th day
Bled - 6th day
Desquam. - 7 day
R. 1455
Inoc. 4. cc citrated blood
Exanthem - 3rd day
Pigmentation - Desquam - 6th day
R. 1456
Inoc. 3. cc. citrated blood
Enanthem - Erythema - 3rd day
Pigmentation - Desquam. - 4th day
Monkey 3
Inoc. 10. cc. defibrinated blood
Leukopenia - Kopliks - Enanthem - 4th day
Exanthem - 5th day
Desquam. - 8th day

Chart 2.—Transmission of measles virus from measles patient Z through three rabbits and three monkeys. (Reprinted by courtesy of The Journal of Infectious Diseases.)

SUMMARY

In the first series of animals the virus of measles obtained from patients with measles on the second day of the disease was passed through 4 rabbits and then produced symptoms typical of measles in a monkey.

In the second series of animals the virus obtained as stated was passed through 3 rabbits and then through 3 monkeys in which symptoms typical of measles were produced. The monkey to monkey passage eliminated any question of rash due to foreign protein.

A control monkey injected with normal human blood showed no reaction.

CONCLUSION

The results of these two series of passages confirm our previous findings that the virus of measles survives rabbit passage and produces symptoms typical of measles in the monkey (M. rhesus).

GROWTH OF B. INFLUENZAE WITHOUT THE PRESENCE OF HEMOGLOBIN

Published in The Journal of Medical Research, 1921, Vol. XLII, pp. 405-417

Anna W. Williams and Olga R. Povitzky

An observation made years ago by Cantani and corroborated by Neisser and others, including ourselves, has recently been denied by Putnam and Gay. The observation is that the influenza bacillus will grow in mixed cultures with certain bacteria without the aid of hemoglobin. Notwithstanding a number of earlier negative reports, positive results had been reported by so many workers and had been obtained by ourselves after so many repeated controlled experiments that we considered that this was one of those questions that might be accepted as settled. Putnam and Gay, however, do not agree with us and neither apparently does Rivers.

The first named authors, Putnam and Gay, do the very thing they criticize in others: namely, they give too few details for us to be sure of the reason why they obtained their negative results.

In the first place, they do not state the age of their cultures at the time of inoculation, neither of the influenza bacillus (which is of special importance), nor of the organisms used for stimulation. Presumably, the age was forty-eight hours, as that is supposed to be the period of maximum growth of most bacteria at 37°C. Anything over that age, according to our experience, is likely to give irregular results with influenza bacilli. We might infer, however, considering their negative results, that these workers used seventy-two hour cultures.

In the second place, they do not state clearly enough the amount of culture they inoculated originally to produce their mixtures. They say that they inoculated rather lightly with the second organism used. We have found in growing certain organisms in mixtures, that unless we inoculate *very* lightly with the stimulating organism in the first culture generation, the influenza bacillus is likely to be overgrown by the accompanying organism.

Another reason for the negative results of Putnam and Gay may be that their culture medium was too alkaline. We have found a reaction of Ph. 7.2—7.4 best for the growth of the influenza bacillus. These authors say that they used a medium varying in reaction from 0.2 to 1.2 acid to phenolphthalein. Just what their end reaction was in each lot of medium we do not know. However, we do know that the reaction of medium is a very important point in relation to the growth of the influenza bacillus. While these bacilli may grow well on certain favorable media at the extremes of reaction, under unfavorable conditions there may be no growth at all at these extremes. We consider 0.2 per cent. acid to phenolphthalein as approaching the extreme towards alkalinity in unfavorable media.

In the fourth place, the medium used may have been so favorable for the stimulating organism that it readily overgrew the influenza bacillus or it may have produced substances deleterious to the influeza bacillus. For example, our cultures mixed with diphtheria bacilli on glucose agar were quite unsatisfactory, while on plain veal agar and on wheat agar the influenza bacilli grew very well in the mixture. Putnam and Gay used glucose agar for their diphtheria and xerosis mixtures.

The authors state that, theoretically, minute traces of blood sufficient for the growth of the influenza bacillus may gain access to the medium through the meat infusion ordinarily employed but if the negative results reported can be relied upon, this can occur seldom, if ever. Putnam and Gay call attention to the fact that Ghon and von Preyss suggested this possibility in 1902. Their idea seems to be that though there would not be enough hemoglobin to allow the growth of the influenza bacillus in pure cultures, a stimulating organism plus the minute trace of hemoglobin would allow growth. Rivers seems to agree with this theory. The interesting work of Thjötta and Avery, elaborating in part the work of Davis, seems to come quite near the solution of the problem of the relationship of hemoglobin to the influenza bacillus. They state that pure crystalline hemoglobin does not by itself support growth but contains only one of the two essential factors for the growth of the influenza bacillus. One factor, "V" is destroyed by autoclaving, the other factor, "X" is not. Avery has other data* which shows that these two factors are found in substances other than a solution of non-autoclaved hemoglobin. His data give evidence also of the nature of these two substances. Whether the bacteria which allow growth contain substances identical with "X" and "V" has not yet been shown.

Experimental Work

Having on hand a number of strains of *B. influenzae* some of which we had isolated during the great pandemic, we thought it might be interesting to repeat our work on mixed cultures of B. influenzae by growing some of these strains with certain organisms on a medium in which no trace of hemoglobin could be present. In order to meet this condition we employed a medium which was made from wheat and contained no meat extract, of any kind. The formula and directions for making are given in the original paper.

This wheat medium was recommended for general laboratory purposes by S. Otabe and was prepared by Dr. Jane Berry of our laboratory for our work. She had made extensive tests of its ability to grow the gonococcus and the other strains of "stimulating" organisms we were using and had pronounced the results ve y good.

In the first series of experiments, three other kinds of mediums were used besides this wheat medium (see table in original article)—nutrient veal agar made according to the formula given in Park and Williams, the same agar plus 1 per cent. glucose sterilized in Arnold, and "chocolate agar" as one control. The final reaction of all these media was not beyond the limits of Ph. 7.4—7.2. The regular transplants were made every forty-eight hours on about 6 cc. of slanted medium in test tubes, 15×3 cm. At the time of each of these transplants additional transplants were also made on Avery's oleate blood agar plates and on veal blood drop plates in order to get surer evidence than could be obtained from stained spreads as to the presence of living influenza bacilli. Avery's medium, as usual, did good service in demonstrating influenza bacilli in mixed cultures with Gram positive bacteria. We had, however, to make the proper dilutions with the cultures containing the yeast-like organisms which we used, because it would readily overgrow the influenza bacilli, making their demonstration difficult if not impossible. The differentiation between the colonies of B. diphtheriae and B. influenzae was best made on the veal-agar-blood-drop plates.

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* Personal communication.

History of strains chosen for this study.—Six strains of B. influenzae were used and five strains of "stimulating" organisms.

Strains of influenza baccilli:

1. "Pembroke,"—from an autopsy case isolated in 1918, as a pure culture from the lungs. It is an individual type by agglutination.
2. "Trahan," another autopsy strain isolated in 1918 and agglutinated individually.
3. 6747,—from a spinal fluid in case of influenzal meningitis in autumn of 1918, agglutinating individually.
4. "Z,"—from another meningitis spinal fluid isolated in 1912. Serologically it belongs to same group as the following two.
5. 747,—from spinal fluid of another meningitis case, isolated in 1912. Serologically it belongs to same group as Z.
6. "B. I. W."—from spinal fluid of case of meningitis isolated a number of years ago by Dr. Wollstein.

Strains used as stimulating organisms:

1. B. diphtheriae,—our No. 8 which we use in making our diphtheria antitoxin, isolated in 1895.
2. "Streptococcus hemolyticus," No. 270, isolated by Valentine in 1917 from a case of mastoiditis.
3. "Gonococcus,"—from one of Torry's strains, "L," used by us in our diagnostic work.
4. "Monilia" (species undetermined),—isolated by one of us in 1920 from the tongue in a case diagnosed as sprue.
5. "Staphylococcus aureus,"—isolated by Valentine from a boil several years ago and used as one of our routine cultures.

We chose the autopsy and meningitis strains of B. influenzae because we had studied them more closely than many of the others. They had all been classed by us as typical influenza bacilli, and they have the following characteristics: All appear to be strict hemoglobinophiles in pure culture; they grow in characteristic colonies on blood drop veal agar plates, and on Avery's oleate agar plates; they have a characteristic morphology in young cultures, namely, they are minute Gram negative bacilli with a tendency to form threads, especially on certain culture media; no one of them is hemolytic; Povitzky found that two of the meningitis strains ("Z" and "747") produce indol; the strains from meningitis sources produce a slight amount of acid in glucose media in forty-eight hours; none produces gas; Strain Z is more virulent for animals than any other strain tested.

For one of the stimulating strains we used B. diphtheriae rather than B. xerosis because in our earlier work B. influenzae in mixtures with this organism had not grown quite so abundantly as with the xerosis bacillus and we wished to make the test as rigorous as possible. For the same reason, we chose a hemolytic streptococcus rather than a pneumococcus or other green-producing streptococcus as in the many times we had made this test with the group of Gram positive streptococci, we had found that all of the stains of influenza bacilli tried, grew better in mass cultures with the green producing streptococci than they did with the hemolytic streptococci which we used. So we again chose the species which had given us the least abundant growths of the influenza bacillus in these earlier experiments. However, we chose the gonococcus as a contrast to the other two because with it we had found that the influenza bacillus grew most abundantly. Cantani was the first to use gonococcus in mixed cultures with influenza bacilli. He discovered accidentally that the gonococcus was a good sensitizing organism and he made some interesting experiments at that time.

We used the monilia because previously we had obtained such good results with yeast, and this monilia was the only yeast-like organism we had growing at this time. (Povitzky succeeded earlier in growing B. influenza with Baker's yeast for many generations.) We chose Staphylococcus aureus as another contrast because we had never obtained growths of the influenza bacillus when put in mixed culture with it, beyond the first few culture generations.

Factors to be considered in carrying out this work successfully: 1. A medium should be used that is not too favorable for the growth of the stimulating organism and that does not produce substances unfavorable to the influenza bacillus. Thus, in our first series of transplants (see table in the original paper) of the mixture of influenza with B. diphtheriae on glucose veal agar, four of the six influenza strains dropped out on the second culture generation and the other two on the seventh and ninth generation respectively, while on plain veal agar all six strains went on growing vigorously through twenty-two culture generations (no more generations tried).

Whether the dropping out of the influenza bacillus from the diphtheria mixtures grown on glucose veal agar, was due to the production of too much acid rather than to a too vigorous growth of the diphtheria bacillus, or to another cause or to a mixture of causes, we do not know. The production of acid is possibly the largest factor. Whatever the cause may be it was probably the same as that giving Putnam and Gay's negative results with their diphtheria and xerosis mixtures.

2. The second factor to be considered in making these transplants is the converse of the above, that is, the medium must be favorable enough for the stimulating organism. For example, when this first series was started the wheat medium used grew the gonococcus quite well through the fourth culture generation. At this time that particular lot of wheat medium was nearly used up and another was employed which gave such a scanty growth that none of the mixtures grew on it after the second or third culture generation. This lot was made during Dr. Berry's absence and the directions could not have been followed absolutely. When Dr. Berry returned, she made up fresh medium which gave most successful growths. With this we started another series of mixtures the results of which are given in the parentheses in the table. We had saved enough of the original medium to carry the mixture of B. diphtheriae, and B. influenzae (strain "Trahan") through twenty-two culture generations or up to the time this series was stopped.

3. The third factor to be considered is the amount of each culture transferred at the first transplant when the mixtures are made. After the first transplant it does not seem to make any difference, within the limits tried, how much or how little of the mixtures is transferred, but when the two cultures are first put together if more than an extremely small amount of certain of the stimulating organisms is used they are likely to overgrow the influenza bacillus. For this reason, we inoculated the gonococcus and the hemolytic streptococcus with a small platinum loop (2 mm.), whereas we transferred the diphtheria bacillus, the monilia and the staphylococcus by touching only the tip of a fine platinum needle to the culture to be transferred. Even with this small amount, the staphylococcus seemed to overgrow the influenza bacillus quickly.

4. The fourth factor is the age of the cultures when transferred. As a rule, all of our cultures were forty-eight hours old when transplanted. Younger cultures we did not try. If the influenza cultures were over forty-eight hours old when transplanted for the mixtures, they were more easily overgrown by the stimulating organism and they seemed unable to recover

their original vigor. For example, on repeating successive transplants on plain veal agar of a mixture of B. influenzae (using three strains respectively) with B. diphtheriae, the strain B. influenzae (Trahan) which had grown the best in the other series, grew much less vigorously than in the previous mixtures. This strain when transplanted for this second series was seventy-two hours old, whereas the companion cultures were forty-eight hours old just as in the first series.

The following observations may be made on this work:

1. Certain strains of B. influenzae grew well on nutrient veal agar in mixed cultures with each of the stimulating organisms tried except the staphylococcus. All grew well with the diphtheria bacillus and the gonococcus. All but one grew well with the monilia. Three only grew well with the streptococcus. These influenza strains did not grow in pure cultures when planted on this same veal agar neither did they grow on this agar when extracts of the gonococcus were added to it (see below), so presumably this medium had none of the "X" factor of Avery and Thjötta.

2. On glucose veal agar, contrary to the above, B. influenzae continued to grow through the entire series only in combination with the gonococcus. With the monilia, the succession continued nearly to the end but with all of the other stimulating organisms the influenza strains dropped out with varying quickness as indicated in the table in the original article. We have mentioned the possible causes of this lack of continued growth.

3. On wheat agar, as we have already said, when the medium was prepared correctly, the influenza bacilli grew abundantly with both diphtheria bacilli and gonococci. Presumably they would have done the same with the streptococcus and monilia; but, judging from the way they acted on other media, no continued growth with staphylococci could be expected.

4. On chocolate agar (which was considered one of the controls) it is interesting to note that while the influenza bacilli seemed completely to overgrow the gonococcus and tended to overgrow the diphtheria bacillus, they were more or less quickly overgrown by the streptococcus and the staphylococcus. In our previous work we had obtained the same results with the pneumococcus as with the gonococcus, that is, the pneumococcus disappeared from the mixture after a certain number of culture generations. Under all the conditions so far tried, the influenza bacilli have disappeared from the staphylococcus mixtures very early. That living staphylococci may contain the factors necessary for the growth of the influenza bacillus is possible, and more experiments may show that the lack of growth is due to an inhibiting factor rather than to an absence of the required factors.

5. Not only do we get varying results on the different mediums with the different stimulating organisms but on the same mediums with the same stimulating organism we get somewhat varying results with the different strains of influenza bacilli. Thus, in the first series of transplants Trahan grew more abundantly on all the mediums used than did any other strain, while "Z" was the poorest grower and the other two strains showed variations between these extremes. These variations were shown chiefly in the mixtures with streptococcus and to a less extent with the monilia.

6. The agglutinating properties of the strains of B. influenzae remained unchanged after their successive transplants in mixed cultures.

Attempts to grow B. influenzae with killed cultures of the stimulating organisms or with their extracts.—Cantani and Luerssen claim to have grown B. influenzae on blood-free mediums with killed cultures of bacteria. Neither of them mentions the number of culture generations through which the growth was carried on. Neisser, on the contrary, though he reported the successful growing of B. influenzae with living cultures of B. xerosis on blood-free agar through twenty culture generations, said he could not corroborate Cantani's work with killed cultures. He assumed, therefore, that a special fermentative assimilation of the foodstuffs by the living organism during its growth is essential for the stimulation of the growth of B. influenzae on blood-free mediums. He gave the name "Amme" (wet nurse) to the stimulating organism.

If neither of the two factors said by Thjötta and Avery to be necessary for the growth of the influenza bacillus is contained in the blood-free nutrient media used by us and if dead bacteria or their extracts contain only one of these factors, as Thjötta and Avery state, then no growth could be expected on a medium made up of a blood free medium and dead bacteria or their extracts.

First experiment.—Monilia and gonococci were killed at different temperatures. The first emulsions of killed organisms tried were prepared as follows:

Forty-eight hour cultures of gonococci or of monilia grown on wheat agar were washed off with salt solution (0.85 per cent.) and the emulsion was heated at 60° C. for one, two, or three hours. The emulsion was then incorporated with the melted wheat agar in the tubes, cooled down to 60° C. Some tubes were poured into plates, others were slanted.

These tubes and plates were then inoculated with B. influenzae.

Extracts of gonococci or of monilia were added to half the tubes and plates on the surface together with the culture of B. influenzae. Proper controls of tubes and plates were made with or without the corresponding extracts to test the sterility of the latter.

The extracts were prepared as follows:

A forty-eight hour growth of gonococcus or of yeast on blood free medium was washed off with distilled water and shaken in an electric shaker for four to five hours. Half of this emulsion was placed in the ice box and half in the incubator at 37° C. over night. The next morning the emulsions were filtered through a Berkefeld filter and tested for sterility.

As a result of these efforts, growth of B. influenzae was obtained only in those instances when the controls proved that the stimulating organism had not all been killed.

In the second experiment the emulsion was prepared as follows:

Forty-eight hour cultures of monilia or of gonococci on wheat agar medium were autoclaved. Some of the tubes were then slanted again and others were poured into plates. The tubes and plates were then inoculated on the surface with B. influenzae with or without the corresponding extracts.

No growth was obtained on any surface.

In the third experiment tried the procedure was the same as in the second one except that the cultures were arnolded instead of being autoclaved.

The results were negative.

The fourth experiment was varied as follows:

A forty-eight hour growth of gonococcus or monilia on wheat agar slants in potato tubes was heated in a water bath at 60° C. for one hour, then inoculated with B. influenzae with or without the corresponding extracts.

The immediate results were not certain. The influenza bacillus was too much masked by the original growth on the surface. The transfers were carried on for three culture generations. At the end of these transplantings Avery's plates were negative for B. influenzae.

In order to ascertain whether or not a ferment or other substance capable of causing growth was secreted in the medium during the growth of the stimulating organism the fifth experiment was made as follows:

Forty-eight hour growths of gonococcus or monilia on wheat agar were removed by washing off with salt solution (0.85 per cent.) and the same tubes were inoculated with B. influenzae, with or without the corresponding extract.

The results were negative.

These negative results with dead cultures and their extracts are corroborative of all our earlier results in our efforts to grow influenza bacilli with dead cultures of micro-organisms. They show that at least one of the substances necessary for the growth of the influenza bacilli had been made inactive by our method of killing the stimulating organism.

Along with these attempts to grow the influenza bacillus on blood free medium with dead bacteria or their extracts we fully corroborated the work of Thjötta and Avery in regard to the growth of these organisms with pure hemoglobin or with autoclaved blood, in fluid mediums. That is, pure hemoglobin in any nutrient medium tried does not support the growth of the influenza bacillus. Neither does autoclaved blood alone support the growth in such medium. But when extracts of certain micro-organisms are added to the medium containing either of these two substances growth of the influenza bacillus is supported indefinitely on successive transplants.

Whether the substances necessary for the growth of the influenza are found elsewhere than in blood and in certain living micro-organisms is a question which has already been solved by Avery (personal communication) and may be more fully worked out in the near future, since a number of investigators are at work on the subject.

CONCLUSIONS

Influenza bacilli may continue to grow in mixed mass cultures with certain micro-organisms on the surface of certain media containing no hemoglobin, through an indefinite number of successive culture generations.

They will not grow on such media with cultures of the same stimulating organisms killed by the method we employed, or with the extracts of these organisms.

For bibliography see the original article.

ANNOTATION: We fully corroborate the work of Avery; namely, of growing B. influenzae in broth containing small pieces of sterile fresh potato. This growth was continued for 12 to 15 generations and then discontinued. The same results were obtained with beets and potato arnolded for 10 minutes. These same vegetables failed to support growth when heated 15 to 20 minutes. Our results in growing B. influenzae with autoclaved potato to which an extract of yeast was added were, on the other hand, variable.

IMPROVED METHODS FOR THE ISOLATION AND LATER CULTIVATION OF B. PERTUSSIS

Published in The Journal of Infectious Diseases, 1923, Vol. XXXII, pp. 8-21

Olga R. Povitzky

The need for more strains of B. pertussis for inquiry as to the presence of types and the possibility of making a more effective vaccine was the starting point of this study. It was difficult to obtain material in the beginning because there were not many cases of whooping cough in New York City during the winter of 1921-1922. Those reported were usually too advanced for the isolation of the organism. We were, therefore, on the lookout for secondary cases in the families in which pertussis had been reported, but in most instances we were able to obtain sporadic cases only.

Bordet and Gengou emphasize the fact, corroborated since by many, that it is a compartively easy task to isolate B. pertussis in the early stages of the disease. In the first ten days under favorable conditions, an almost pure culture of B. pertussis is frequently obtainable.

In general, the favorable factors include: (1) a sputum which has been obtained at the end of the characteristic spasmodic cough; (2) the washing of the sputum a few times in salt solution, after which a suspension for plating is made of any characteristic balls present; (3) freshly prepared plates of Bordet-Gengou medium made with defibrinated or citrated blood in the proportion of 1:3. The blood must be added at a temperature of from 45 to 50 C. and well mixed with the agar so that the plates will appear smooth, uniform and of a proper red color.

It is hard to get sputum from young children unless it is expelled in a fit of spasmodic coughing, or is removed from the mouth or throat with a spatula by depressing the tongue before the child has had a chance to swallow after the coughing spell.

The medium in use in this laboratory for isolation of B. pertussis is that of Bordet-Gengou modified slightly in a few points: thus 2% instead of 1% glycerol is added; horse defibrinated or citrated blood instead of human or rabbit blood is used. The original formula of Bordet-Gengou does not give the reaction of the medium. Looking over the literature on the isolation of B. pertussis, we find that Arnheim is the only one who mentions the reaction which he says is feebly acid, but he does not say which indicator he used to ascertain this point. The unadjusted Bordet-Gengou medium is feebly acid to litmus, but when tested as to the H-ion concentration, it is found to be Ph 5.8 to 6.1, that is, rather acid. In our laboratory the medium was originally adjusted by adding 8 cc. of normal sodium hydroxid to each liter; this made the reaction about neutral to phenolphthalein before sterilization and supposedly slightly acid after sterilization. Williams, Wilson and Mishulow adjusted their Bordet-Gengou medium to this reaction for the isolation of B. pertussis in 1912 (personal communication). Recent tests of such a medium have shown that it may vary in reaction from Ph 7 to 7.8; usually it is about 7.4 to 7.6. In the beginning of the present work, we used a Bordet-Gengou medium adjusted to 7.2 to 7.4.

TECHNIC

From 6 to 8 plates of Bordet-Gengou medium and 3 "chocolate" plates made with Avery's sodium oleate agar were used for each case. The latter were useful for the purpose of determining the percentage of B. influenzae present. The Bordet-Gengou plates were incubated for from 48 to 72 hours and occasionally for 4 days. The colonies picked for fishing into Bordet-Gengou tubes were the minute whitish colonies, preferably those which showed a lightened area of the surrounding medium. In 48 hours, smears were made from all pertussis-like cultures (usually a whitish nonspreading growth), stained by gram and examined under the microscope. If the smears showed small ovoid, uniform gram-negative rods, the culture was transplanted to both chocolate and plain agar. If no growth occurred on either of these agars and if at the same time a good growth occurred on the Bordet-Gengou medium, the growths were considered culturally B. pertussis. They were then turned over to Miss Mishulow for further identification and typing with known pertussis serums.

In most cases from which B. pertussis was isolated the culture was obtained pure at the first attempt, but occasionally it was necessary to replate it a second or third time.

For these cases Bordet-Gengou medium (reaction PH 7.2 to 7.4) was used exclusively. In this series we succeeded in obtaining 8 isolations of B. pertussis. The period of the disease in which the successful isolations occurred varied from the first to the third week of the whoop: in the first week, 3 isolations of 7 cases; in the second, 4 of 14 cases; in the third, 1 of 7 cases.

The cases were usually in the postcatarrhal stage. The statements of the patient's family were not always reliable, for the parents were not clear as to the distinction between the beginning of the cough and the beginning of the whoop.

This series comprised uncomplicated whooping cough only. Cases complicated with pneumonia, bronchopneumonia and exanthems are not included. Neither are any suspicious cases in which the patient developed a cough after a course of prophylatic treatment with pertussis vaccines. These children did not prove to have pertussis eventually. In neither of these groups was B. pertussis isolated.

FIRST SERIES; 35 CASES (TABLE 1)

TABLE 1

Isolation of B. Pertussis on Bordet-Gengou Medium, Reaction Ph 7.2-7.4

First Series of Thirty-Five Cases

Number of Cases	Week of Whoop	Number of Isolations
7	1	3
14	2	4
7	3	1
7	4	0

The percentage of successful results obtained with our first series, namely, 8 isolations from 35 cases (over 22.8%) correspond more or less with the results of most other workers, with a few exceptions (see table 2). In contrast to the majority of workers, Menschikoff reported almost 100% of isolations up to the eighth week of the disease; Inaba from 88 to 91% Seiffert, early cases, 75%; Chievitz and Meyer, from 53 to 41%. The first two workers do not state whether they verified their cultures by serologic tests for complete identification.

TABLE 2

Tabulation of Isolations of B. Pertussis Reported by Various Authors

Author and Year	Number of Whooping-Cough Cases	Stage	Isolations	
			Number	Percentage
Arnheim, 1908	20	Not mentioned	6	30
Fraenkel, 1908	38	Not mentioned	8	21
Seiffert, 1909	16	Early	12	75
Wollstein, 1909	20	From catarrhal to seventh week	5	25
Williams, 1912	187	From first to fourth week	27	14½
Poleff, 1913	14	Not mentioned	3	14½
Odaira, 1911	42	Not mentioned	5	12
Inaba, 1912	78	Early	68	88
Menschikoff, 1909	94	From beginning till eighth week	93	Nearly 100
Klimenko, 1908	76	....	5	6½
Chievitz and Meyer, 1916 (1st series)	126	From catarrhal to fifth and sixth weeks	67	53
Chievitz and Meyer, 1916 (2nd series)	165		69	41.8
Shiga, 1913	Not given	Not mentioned	10	26.3
Giese, 1918	19	Not mentioned	5	

Menschikoff used Bordet-Gengou medium to which hemoglobin in the proportion of 1:3 was added. The hemoglobin was obtained by freezing and thawing the blood and keeping it at a temperature above 0 C. for 24 hours.

Inaba's medium was a plain 3% agar solution to which difibrinated goat blood was added, 1:4. He does not mention whether he used salt or water with the agar and omits reference to the reaction.

Shiga isolated 10 cultures of B. pertussis; he does not state from how many cases. His medium was a modified Bordet-Gengou, that is, 1% peptone was added and the reaction made slightly alkaline (to what?). After the blood was added, the tubes or plates were heated at 56 C. for one-half hour. This medium is so favorable for the isolation of B. influenzae that it is hard to understand how Shiga obtained pure cultures of B. pertussis. Perhaps this is the reason why this worker thinks that the two organisms are closely related and belong to one group.

Arnheim isolated 6 pertussis cultures from 20 cases and Frankel 8 from 38 cases. Frankel also reports that he isolated B. pertussis from 2 cases which were not pertussis clinically. Wollstein had 5 isolations from 20 cases. Williams, Wilson and Mishulow had 27 isolations from 187 cases (14.5%) [unpublished], Poleff 3 isolations from 14 cases, Odaira 5 isolations from 42 cases, Klimenko 5 isolations from 76 cases, and Giese 5 isolations from 19 cases.

An analysis of the 8 isolations in our first series, brings out some facts which are interesting, for they illustrate the importance of the stage of the disease in attempts to isolate the bacillus.

The first culture of B. pertussis was isolated from a child of 18 months. A sister of 5 years and a brother of 6 were reported to us as having whooping cough. These 2 children had been whooping for over 2 weeks and the results with the sputum were negative. On a visit to the home of this family, it was discovered that the little sister of 18 months had just started to whoop. There was no difficutly in isolating the organism from the sputum. Of 5 cases in a school for colored children, B. pertussis was obtained in the case of one child only—a boy of 7. On a visit to the family of one of these children, a child of 2 was found to be coughing and had just begun to whoop.

This case had not been reported to us. The sputum of this child gave us our third B. pertussis culture.

The fourth case which gave positive results was reported as suspicious whooping cough chiefly because the sister had the disease in a late stage. In the case reported, that of a girl of 10, there was a dry cough. No culture was obtainable by the droplet method recommended by Chievitz and Meyer. This method consists of making the patient cough on a Bordet-Gengou plate held a few inches from the face. In a few days the dry cough loosened up and an almost pure culture of B. pertussis was obtained from the sputum seeded on Bordet-Gengou medium.

The fifth culture was isolated from a boy of 3 years. No sputum could be obtained in the first attempt. Three days later sputum mixed with fresh vomit was brought to us; from this material we succeeded in isolating B. pertussis. A second culture taken one week later from this patient proved to be negative. The sixth and seventh cultures of B. pertussis were obtained from children in the Bellevue Clinic by the courtesy of Dr. Chaplin. One child was in the first stage of the disease, the second in the third week. The sister and brother of the first child who were in more advanced stages of the disease gave negative results. The eighth and last culture of this series was obtained from 1 of the 4 children examined in a home for children where there was an epidemic of whooping cough.

The greatest obstacle in isolation of B. pertussis was the accompanying growth of B. influenzae. The latter organism was isolated from almost every case of pertussis; it was always present in great numbers at the beginning of the second week of the whoop. The Bordet-Gengou medium with a reaction of Ph 7.2 to 7.4 is favorable for the growth of B. influenzae. The colonies develop in from 18 to 24 hours and thus outgrow the B. pertussis colonies, for the latter are not apparent until the third or perhaps the fourth day of incubation. The colonies of B. influenzae are very much like those of B. pertussis, except that they do not show a light colored area in the blood plate. When, however, the colonies of B. influenzae happen to be of the hemolytic variety even this diagnostic difference disappears.

Experimental Work.—In view of the foregoing facts the chief object in the search for a more favorable medium for isolation of the Bordet-Gengou bacillus was to find a substance which would inhibit in a measure the development of the colonies of B. influenzae and at the same time be favorable for the growth of B. pertussis.

The first attempts at modification consisted in the addition of various dyes, such as gentian violet, methylene blue, methylene green, brilliant green, carmen borax and others. These were tried in different dilutions with pure cultures of B. pertussis and B. influenzae. The results were not satisfactory. The majority of these dyes acted alike on these two organisms, while a few even enhanced the growth of B. influenzae. Finally, when Grübler's acid green was tried the results were more encouraging. With a dilution of 1:20,000 of this dye, the growth of B. pertussis was much better than that of B. influenzae. The suggestion of an acid reaction in acid green prompted us to make comparative tests with the 2 organisms on a potato-glycerol-veal agar with the reaction unadjusted, and, therefore, quite acid (Ph 5.8 to 6.1). We are accustomed to use this medium for the growth of B. mallei. Citrated horse blood in proportion of 1:4 was added to the medium at a temperature of 45 C., well mixed with the agar and slanted. B. pertussis and B. influenzae were inoculated in companion tubes of this medium. The tube inoculated with B. influenzae showed almost no growth after 48 hours' incubation. The growth of B. pertussis, on the other hand, was astonishing; so much so, that at the first glance, it was thought a contamination. Only a smear and a subsequent plating convinced us that we were dealing with a pure culture of B. pertussis. On this medium the Bordet-Gengou bacillus grows in a brittle pearly white mass.

The next procedure was to make comparative plate tests of B. pertussis, B. influenzae and a mixture of both in 4 mediums, 2 of these being unadjusted and therefore of an acid reaction. These mediums were: (1) Bordet-Gengou medium adjusted to Ph 7.2 to 7.4; (2) Bordet-Gengou medium unadjusted, Ph 5.8; (3) potato-glycerol-veal agar plus blood unadjusted, Ph 6, and (4) chocolate agar, Ph 7.2 to 7.4.

Uniform suspensions of the same density (rather light) were made from 48-hour cultures of B. pertussis grown on Bordet-Gengou medium and B. influenzae grown on chocolate agar; also of a mixture of both organisms. The same sized loop was used for streaking all plates. Further dilution of the suspension was made by inoculating 3 plates in succession with the same loopful.

Table 3 shows how favorably B. pertussis grows on the acid mediums as compared with B. influenzae; while on the Bordet-Gengou medium adjusted to Ph 7.2-7.4, the B. influenzae grows almost as well as B. pertussis. On chocolate agar the freshly isolated B. pertussis does not grow, while B. influenzae gives its usual luxuriant growth.

TABLE 3.

COMPARATIVE GROWTH OF B. PERTUSSIS AND B. INFLUENZAE AND A MIXTURE OF BOTH ON STRONGLY ACID AND FEEBLY ACID BORDET-GENGOU MEDIUM AND ALSO ON ACID GLYCEROL-VEAL-POTATO AGAR PLUS BLOOD

Plates			Bordet-Gengou Medium		Glycerol-Veal-Potato Agar Plus Blood Ph 6.0	Chocolate Agar Ph 7.2-7.4
			Ph 7.2-7.4	Unadjusted Ph 5.8		
B. influenzae	1		++	—	+	+++
	2		±	—	+	++
	3		—	—	±	+
B. pertussis	1		++	+++	+++	—
	2		++	+++	++	—
	3		±	+++	+	—
Mixture of B. influenzae and B. pertussis	1	B. influenzae	+	—	+	+++
		B. pertussis	+++	+++	++	—
	2	B. influenzae	+	—	±	+++
		B. pertussis	++	+++	+	—
	3	B. influenzae	—	—	—	+++
		B. pertussis	±	+++	±	—

In the mixture of B. influenzae and B. pertussis on the acid mediums, the preponderance of B. pertussis was made conspicuous by the areas of lighter color surrounding the colonies. There was a good growth of B. influenzae on Bordet-Gengou medium with a reaction of Ph 7.2 to 7.4 and much less growth of B. pertussis, as indicated by a smaller number of colonies showing the lighter areas on the medium.

The next attempt was to find the limits of acidity favorable for the growth of B. pertussis. A series of careful preliminary tests was made to determine the Ph values of the mediums after the addition of different amounts of various acids. It was thought best to use organic acids for this purpose. Small amounts of acetic, lactic and tartaric acids were added to Bordet-Gengou and to potato-glycerol-veal agar in quantities sufficient to make the final dilutions from 1:2,500 to 1:100. The mediums were then tested as to their reaction.* These tests were made before the addition of blood, and the reaction may, therefore, have been changed slightly by the blood. On the advice of C. E. Ekroth, potassium oxalate was also tried. Though in itself it is neutral, the medium to which it is added becomes more acid. The results of this test as to acidity are shown in table 4.

TABLE 4.

REACTIONS OF MEDIUMS ADJUSTED TO DIFFERENT H-ION CONCENTRATIONS BY THE ADDITION OF VARIOUS ORGANIC ACIDS OR OF POTASSIUM OXALATE

Medium	Acid	Final Dilution 1:2,000 Ph	Final Dilution 1:1.000 Ph	Final Dilution 1:500 Ph	Final Dilution 1:100 Ph
Bordet Gengou	Lactic	4.4	4.1	3.7	3.2
Glycerol-potato-veal agar	Lactic	5.4	5.0	4.3	3.6
Bordet Gengou	Acetic	4.45	4.4	4.1	3.7
Glycerol-potato-veal agar	Acetic	5.3	5.0	4.4	4.1
Bordet Gengou	Tartaric	4.2	3.9	3.6	3.2
Glycerol-potato-veal agar	Tartaric	5.5	4.4	4.3	3.6
Bordet Gengou	Potas .oxalate*	4.4	4.5	4.5	4.4
Glycerol-potato-veal agar	Potas .oxalate*	4.4	4.5	4.4	4.5

* Only one sample marked "technical, neutral" gave us the reactions recorded in this table. The results in isolation continued to be satisfactory with the dilution of 1:2,000. Later tests of this sample, when added to the Bordet-Gengou medium, showed, however, a much less acid reaction (Ph 6.1 to 6.3) as did also other samples marked "tested purity" and "blue label reagent, neutral."

It is seen by this table that the same dilutions of the three acids have a more acid effect on the Bordet-Gengou medium than on potato-glycerol-veal agar. This is probably because the Bordet-Gengou medium contains no meat and has therefore practically no buffers.

Thus, while the reaction of potato-glycerol-veal agar after addition of each of the 3 acids in 1:2,000 dilution is from Ph 5.5 to 5.2, the reaction of Bordet-Gengou medium in the same dilution is from Ph 4.4 to 4.2. In a dilution of 1:1,000, the reaction of potato-glycerol-veal agar is from Ph 5 to 4.4; that of Bordet-Gengou is from 4.4 to 3.9. In 1:500 dilution, the reaction of potato-glycerol-veal agar is from Ph 4.4 to 4.3; that of Bordet Gengou from 4.4 to 3.6. In 1:100 dilution, the reaction of potato-glycerol-veal agar is from Ph 4.1 to 3.6, while that of Bordet-Gengou is from 3.7 to 3.2. Potassium oxalate, on the other hand, seems to act on both mediums alike, and the reaction is the same (Ph 4.5 to 4.4) in all 4 dilutions.

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*The determinations were made by Mr. A. M. Last, chemist in the Division of Media. The colorimetric method was used.

To prepare these mediums it was found necessary to add the acids in suitable dilution to the medium after sterilization, just before addition of the blood. If the acids are added to the mediums before the autoclaving, the agar tends to lose its solidifying property. This is true especially when the stronger dilutions of acid are used. Agar containing dilutions of acid as high as 1:2,500 or 1:3,000 can be sterilized with little effect on its firmness. Since 1922 all sterilization of Bordet-Gengou medium has been carried out in the Arnold sterilizer for one hour on each of three successive days.

In making tests as to the limits of acidity favorable for the growth of B. pertussis, we confined ourselves entirely to the Bordet-Gengou medium, for reasons which will be given later.

Table 5 shows the limits of growth of pure cultures of B. pertussis on Bordet-Gengou medium with the different acids and potassium oxalate. The results as summarized are as follows:

Acetic Acid.—Growth is good on Bordet-Gengou medium with the addition of this acid in 1:2,000 dilution (PH 4.45); growth also good in 1:1,000 dilution (PH 4.4); growth is poor in 1:500 dilution (PH 4.1); no growth occurred at all with 1:100 dilution (PH 3.7).

TABLE 5

FORTY-EIGHT HOUR GROWTH OF B. PERTUSSIS ON BORDET-GENGOU MEDIUM ADJUSTED TO VARIOUS H-ION CONCENTRATIONS WITH DIFFERENT DILUTIONS OF VARIOUS ORGANIC ACIDS OR OF POTASSIUM OXALATE

Acid	Final Dilution 1:2,000 PH	Growth	Final Dilution 1:1,000 PH	Growth	Final Dilution 1:500 PH	Growth	Final Dilution 1:100 PH	Growth	Control Acid Added Without Blood
Acetic	4.45	++	4.4	++	4.1	±	3.7	—	—
Lactic	4.4	++	4.1	++	3.7	+	3.2	—	—
Tartaric	4.2	++	3.9	++	3.6	+	3.2	—	—
Potassium oxalate..	4.4	++	4.5	++	4.4	++	4.5	++	—

Lactic Acid.—Growth is good with 1:2,000 dilution (PH 4.4); also good with 1:1,000 (PH 4.1); fairly good with 1:500 dilution (PH 3.7); no growth with 1:100 dilution (PH 3).

Tartaric Acid.—Growth is good with 1:2,000 dilution (PH 4.2); also good with 1:1,000 (PH 3.9); fairly good in 1:500 dilution (PH 3.6); no growth in 1:100 dilution (PH 3.2).

With potassium oxalate, the growth is good with all 4 dilutions, namely, from 1:2,000 to 1:100 dilution. The primary dilution of potassium oxalate was made by adding 1 gm. to 10 cc. of distilled water, which made a 1:10 dilution. The same proportion was observed with tartaric acid.

With pure cultures of B. pertussis, the growth on a medium to which acid had been added was, as a rule, good as long as the blood in the medium remained bright red. This was usually the case with the mediums to which acids in dilutions of 1:2,000 or 1:1,000 were added. When the dilutions were 1:500 and lower, the medium turned brown and little or no growth of B. pertussis occurred. With potassium oxalate the medium remained red with all 4 dilutions.

ISOLATION OF B. PERTUSSIS IN A SECOND SERIES OF CASES ON AN ACID MEDIUM

A second series of isolations was undertaken to test the worth of the following mediums for isolation purposes: Potato-glycerol-veal agar plus blood, chocolate agar, Shiga medium and Bordet-Gengou medium.

Potato-glycerol-veal agar plus blood, on which B. pertussis in pure culture grows so luxuriantly immediately after isolation, was found not suitable for the actual isolation of the organism. The plates were invariably overgrown, because this medium is too favorable for the growth of the associated organisms of the mouth and nasopharynx.

Chocolate-veal agar had been used first by the writer in 1912-1913 in work on influenza, and later for pertussis; it was then referred to as "coagulated blood veal agar." This medium is not suitable for isolation because B. pertussis grows in it only after preliminary cultivation on Bordet-Gengou for a number of weeks, and the organism must become accustomed to the modified blood. After that, the growth becomes luxuriant.

The Shiga medium as described is not suitable because it also is too favorable for the growth of B. influenzae and the other organisms usually present in the sputum of pertussis patients.

Bordet-Gengou medium, which is made without meat or peptone, is less favorable for the growth of the associated organisms of the mouth and nasopharynx. This medium (of a reaction PH 6.1 to 5.0) was the best for purposes of isolation and it was, therefore, the medium selected for our further tests.

The reaction of the mediums for the second series of isolations were as follows:

1. Bordet-Gengou adjusted with normal NaOH to PH 7.2 to 7.4 (used exclusively in the first series of isolations).
2. Bordet-Gengou unadjusted, PH 5.8 to 6.1.
3. Bordet-Gengou adjusted with acetic or lactic acids to PH 5.
4. Bordet-Gengou with acetic, lactic, tartaric acids in 1:2,000 and 1:1,000 from PH 4.5 to 3.9.
5. Bordet-Gengou with potassium oxalate in 1:2,000 and 1:1,000 dilutions.
6. Chocolate-veal agar, PH 7.2 to 7.4.

Of the modifications described under 4, only Bordet-Gengou with lactic acid added to 1:2,000 dilution was retained. As mentioned before, B. pertussis grows well in pure culture with each of the 3 acids added in dilutions of 1:1,000 to 1:2,000. This is not true, however, when the same mediums are used for purposes of isolation. It was found that when the acids were added in the proportion of 1:1,000 dilution, all of the plates turned brown the next day and could not be used. When the acids were added in 1:2,000 dilution, the results were variable. In this dilution, the lactic acid proved better than either the acetic or tartaric acids in the same dilution. The most constant results were obtained with agars containing acetic and lactic acids, PH 5 (1:2,500 dilutions); with potassium oxalate 1:2,000 and occasionally in 1:1,000 dilutions.

Table 6 shows the superiority of the acid mediums over the medium with a reaction of PH 7.2 to 7.4. With the Bordet-Gengou medium of the latter reaction in the first series of cases, we succeeded, as stated, in isolating 8 cultures from a total of 35 cases, over 22.8%. In contrast to this figure, our second series of cases tested at the same stages of the disease with the same medium, but of acid reaction, showed over 43.5% isolations (10 in 23 cases).

TABLE 6.

ISOLATION OF B. PERTUSSIS ON ACID BORDET-GENGOU MEDIUM REACTION PH 6.1 TO 4.4

Second Series of Twenty-Six Cases

Number of Cases	Week of Whoop	Number of Isolations	Percentage of Isolations
7	1	5	over 71.4
7	2	3	about 43.0
6	3	1	about 17.0
3	4	1	over 33.0
3	5	0	

In the second series, there were really 26 cases including the three in the fifth week of the whoop. Of the 26 cases, 24 came from the home for children. In the majority of cases obtained from the home for children, there were 2 unfavorable factors: The first was the early age of the children, from 2 to 4 years, at which period sputum is obtained with difficulty; the second was that the nature of the disease was not recognized until the symptoms were more or less advanced and past the catarrhal stage. Under more favorable conditions than these, a still higher percentage of isolations with the acid medium could be expected.

Table 7 gives still further proof of the advantage of an acid reaction. Thus with Bordet-Gengou medium, reaction Ph 7.2 to 7.4, only 4 of 23 cases gave cultures of B. pertussis. With the unadjusted Bordet-Gengou (Ph 6.1 to 5.8) 7 of 23; with the Bordet-Gengou adjusted to Ph 5 with acetic acid, 10 cultures were obtained. Bordet-Gengou adjusted with potassium oxalate in 1:2,000 dilution (reaction? see note to table 4), 10 positive cultures from 23 cases up to the fourth week of the whoop. As seen in table 6, no results were obtained from any cases in the fifth week of the whoop.

TABLE 7.

COMPARATIVE VALUES OF DIFFERENT REACTIONS OF BORDET-GENGOU MEDIUM FOR ISOLATION OF B. PERTUSSIS IN DIFFERENT STAGES OF THE DISEASE

Number of Cases	Week of Whoop	Reaction Adjusted with Normal Sodium Hydroxid to Ph 7.2-7.4	Reaction Unadjusted Ph 5.8-6.0	Reaction Adjusted with Acetic Acid to Ph 5.0 (Dilution 1:2,500)	Reaction Adjusted with Lactic Acid to Ph. 4.4 (Dilution 1:2,000)	Reaction Adjusted with Potassium Oxalate to Ph. 4.5 (Dilution 1:2,000)	Total Number of Isolations
7	1	3	4	5	4	5	5
7	2	1	2	3	2	3	3
6	3	0	1	1	0	1	1
3	4	0	0	1	0	1	1
3	5	0	0	0	0	0	0
Total 23	...	4	7	10	6	10	10

One objection to the acid reaction in the Bordet-Gengou medium is the fact that it also favorable for the growth of gram-negative cocci. This difficulty is more than compensated for by the control of the growth of B. influenzae and other organisms.

In order to ascertain whether B. pertussis could be isolated from conditions other than whooping cough, control tests were made, at the suggestion of Dr. W. H. Park. Fifteen sputums from children ill with scarlet fever and measles and various other infections, also three from adults ill with scarlet fever, were tested with the acid Bordet-Gengou medium. No B. pertussis was isolated in any case.

In further attempts to isolate B. pertussis from cases other than whooping cough, cultures were taken from 30 cases of croup, 50 cases of measles, 40 cases of scarlet fever (all patients in Willard Parker Hospital) and from a number of nurses and laboratory workers suffering from colds. In no instance was B. pertussis isolated; B. influenzae, however, was found in a majority of these cases, together with gram negative cocci, green streptococci and streptococcus hemolyticus.

In addition to the proportion of isolations, we were interested in ascertaining the length of time during which B. pertussis could be obtained from a given case.

Attempts were made in a number of cases to obtain repeated cultures; the sputum was obtained in 2 cases 3 times, and in 1 case 4 times. Positive results were obtained in 2 successive cultures from 2 cases only. The third cultures from the same cases were negative. This work is still in progress.[1]

In Copenhagen, according to Chievitz and Meyer, the children are readmitted to school after the fifth week of the disease after the cultures have proved negative twice, in spite of the fact that the children still continued to whoop and cough. The experiments of these two workers in this direction have convinced them that whooping cough is not infectious after the fifth or sixth week of the disease.

An attempt was made in 2 cases to use the medium mentioned by Inaba, but on account of the meager description given by this writer, we did not know whether we were using the same medium or not. We omitted from the unadjusted Bordet-Gengou medium the glycerol and potato extract. It is evident that such a medium would be even less favorable for the associated organisms than the Bordet-Gengou medium. On the other hand, it may lack sufficient nourishment for the development of B. pertussis. This is suggested by the fact that though we obtained positive results on this medium, the colonies were less numerous than on the other mediums used, and on all positive results were given with material from these 2 cases.

The Chievitz and Meyer droplet method mentioned before, was used in six cases of the second series with negative results. These cases were in the second week of the whoop. However, many colonies of B. influenzae appeared on chocolate Avery plates from the same cases by the droplet method.

SUBSEQUENT GROWTH OF B. PERTUSSIS

After isolation, this organism continues to grow well on Bordet-Gengou medium, especially on the unadjusted PH 5.8 to 6.1. It grows best on unadjusted potato-glycerol-veal agar (PH 5.8 to 6.1) plus blood. This medium should be excellent for the cultivation of large quantities of B.

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[1] We have not yet been able to find the pertussis bacillus after the fourth week of the whoop.

pertussis and for the making of antigens and vaccines. The medium which is in use at present in our laboratory for this purpose is chocolate-veal agar. Although B. pertussis grows luxuriantly on this medium, it does not do so until many weeks after isolation, as mentioned before; while on potato-glycerol-veal agar plus blood it grows at once.

B. pertussis grows just after isolation also on Shiga's medium but not as luxuriantly as on the potato-glycerol-veal agar plus blood or on chocolate veal agar, after preliminary cultivation.

SUMMARY

It was found that a definitely acid reaction in a suitable medium is favorable for the isolation and growth of B. pertussis. Such a reaction is especially valuable because it inhibits the growth of B. influenzae and other organisms found in the sputum of patients with pertussis.

The most favorable point of acidity for isolation was found to be at PH 5.

The limits of acidity favorable to the growth of B. pertussis are PH 6.1 to 4.4.

The unadjusted Bordet-Gengou medium for the cultivation of B. pertussis is usually of the reaction PH 5.8 to 6.1. If a more acid reaction is desired, this can be obtained by adding one of several organic acids to a medium, preferably after the latter has been sterilized and before the addition of the blood.

B. pertussis grows immediately after isolation most luxuriantly on potato-glycerol-veal agar unadjusted (PH 5.8 to 6.1) plus blood in proportion of 1:3 or 1:4 added at a temperature of 45 C.; but this medium is too favorable for the growth of various other organisms found in the sputum and is therefore not suitable for the isolation of B. pertussis.

On the other hand, the Bordet-Gengou medium, which contains neither meat nor peptone, is less favorable for the growth of the associated organisms of the mouth and nasopharynx. It was found, therefore, that this medium after adjustment to a suitable acid reaction (PH 5) was the best for the isolation of B. pertussis.

NOTE: Since the publication of the above, twenty more strains of the Bordet-Gengou bacillus have been isolated from sporadic cases of whooping cough, using our modified acid medium. According to the agglutinin absorption test all thirty-eight strains isolated by the writer belong to type B (the type to which the original Bordet-Gengou strain belongs). The percentage of isolation has been very high—from seventy-five to eighty-five per cent.—in the first week of the whoop.

I am indebted to Miss Mildred S. Hollinshead for her assistance in this work.

For bibliography see the original article.

THE EXISTENCE OF MORE THAN ONE IMMUNOLOGIC TYPE OF B. PERTUSSIS

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Charles Krumwiede; Lucy Mishulow, and Carolyn Oldenbusch

In an investigation of the antigenic properties of pertussis vaccine, it became necessary to isolate a new strain of B. pertussis for comparison with older stock strains. Several strains, therefore, were isolated having all the typical morphologic and cultural characteristics of the Bordet-Gengou bacillus. These strains were tested agglutinatively with the serum of a rabbit which had been inoculated with the old stock strain utilized in the vaccine experiments. This serum agglutinated the freshly isolated strains nearly as well as the homologous serum strain. Rabbits were then injected with a suspension of one of the newly isolated strains. This serum to our surprise caused only slight agglutination (1:100) of the old stock strain, whereas the other freshly isolated strains were agglutinated to practically the same degree as the serum strain.

These observations led to a more detailed study of a series of strains of B. pertussis, utilizing agglutination and agglutinin-absorption for differentiation. From various sources we collected a series of stock strains among which was a strain with a history of having been obtained from Bordet. Fresh strains were obtained from 17 cases. For most of these strains we are indebted to Dr. Olga R. Povitzky, who isolated them while working on the improvement of mediums for the isolation and cultivation of B. pertussis.

As may be seen from tables 1, 2 and 3, the strains tested by agglutination and agglutinin-absorption fall into 2 groups, A and B. The tendency of group B is to cross agglutinate with serums of group A., also to absorb a large proportion of the specific agglutinins. Group A. strains, on the other hand, practically do not agglutinate with serums of group B and show little tendency to reduce the specific agglutinins.

TABLE 1

PRELIMINARY GROUPING BASED ON THE RESULTS OBTAINED WITH A LARGE ABSORBING DOSE 1:10

Strains Tested	Serum 253			Serum Bordet P. D.		
	Agglutination			Agglutination		
	Before Absorption	After Absorption		Before Absorption	After Absorption	
		Absorbing Strain	Serum Strain		Absorbing Strain	Serum Strain
Group A						
253	2,000++	0	0	0	0	1,000++
M 254, 1	1,500++	0	0	100++	0	2,000++
217 L	1,000++	—	—	0	0	1,000++
246 L	1,000++	—	—	0	0	1,000++
247 L	500++	—	—	0	0	1,000++
248	1,500++	0	0	0	0	1,000++
250 L	1,000++	—	—	0	0	1,000++
251	1,500++	0	0	0	0	1,000++
252 M	1,500++	—	—	0	0	2,000++
Group B						
Bordet P. D.	1,000++	0	200++	2,000++	0	0
0590 P D	1,000++	0	200++	1,000++	0	0
98 P D*	800++	0	0	1,000++	0	0
110 P D	800++	0	100++	1,000++	0	0
163 P D	800++	0	400++	1,000++	0	0
248 P D	500++	0	200++	1,000++	0	0
248 M	800++	0	400++	1,000++	0	0
M 254,4	1,000++	0	150++	1,000++	0	0
M 255,4	800++	0	400++	1,000++	0	0
M 256,11	800++	0	200++	1,000++	0	0
P 257,5	1,000++	0	100++	1,000++	0	0

* In dose of 1:80 no agglutination of absorbing strain in dilution of 1:25, but marked agglutination of serum strain at 1:400.

In this and the following tables, "Bordet" is a strain obtained from Bordet in 1911.

The numbers below 254 refer to strains isolated in this laboratory 1913-14; letters after the numbers designate laboratories from which the strains, originally sent out from this laboratory, were obtained. Strain 0590 was isolated about 1911.

Strains 254 and higher were isolated between February, 1921, and April, 1922, by Povitzky (P) and Mishulow (M); the number after the comma refers to individual fishings.

In all tests 1:100 is the lowest dilution used in the direct agglutination tests; 1:25 is the lowest dilution used after absorption, and in all cases in which there was no agglutination in this dilution, higher dilutions were without effect also; 1:200++ etc., indicates highest dilution giving marked but partial agglutination.

+++, complete agglutination; ++, marked but partial agglutination; +, slight agglutination; 0, no agglutination; —, not tested.

TABLE 2

CONFIRMATORY RESULTS OBTAINED WITH ANOTHER SERUM, USING A LARGE ABSORBING DOSE 1: 10

Strains Tested	Serum 251: Agglutination		
	Before Absorption	After Absorption	
		Absorbing Strain	Serum Strain
251	4,000++	0	0
253	4,000++	0	0
M 254,1	6,000++	0	0
0590 P D	1,000++	0	3,000++
98 P D	1,000++	0	3,000++
110 P D	2,000++	0	3,000++
163 P D	1,000++	0	3,000++
M 254,4	600++	0	1,500++
M 255,4	2,000++	0	100++
M 256,11	1,000++	0	1,500++

Other confirmatory tests were made with serum M 255,4, testing 16 strains, and with serum M 254,4, testing 11 strains. The results with these serums were so closely like those obtained with serum Bordet (table 1) that there seems to be no reason for publishing the tabulation of the results.

TABLE 3

ABSORPTION OF SERUM "BORDET" GROUP B. BY STRAINS OF GROUP B., ABSORBING DOSE, 1: 200

Strains Tested	Agglutination Before Absorption	Agglutination After Absorption	
		Absorbing Strain	Serum Strain
Bordet P D	2,000+	0	0
0590 P D	1,000++	0	0
98 P D	2,000+	0	0
110 P D	2,000+	0	0
163 P D	2,000+	0	0
248 P D	1,000++	0	0
248 M	1,000++	0	0
249 L	1,000+	0	0
M 254,3	1,000++	0	0
M 255,1	1,000+++	0	0
M 256,1	1,000++	0	0
P 257,5	2,000+	0	0
P 258,1	1,000++	0	0
P 259,1	2,000+	25+	0
P 260,1	2,000+	0	0
P 261,1	1,000++	0	0
P 262,8	2,000+	25++	25++
P 263,5	2,000+	25+	25+
P 264,4	2,000+	25+	25+

TABLE 4.

ABSORPTION OF SERUM 253 GROUP A BY STRAINS OF GROUP A. ABSORBING DOSE, 1: 200

Strains Tested	Agglutination Before Absorption	Agglutination After Absorption	
		Absorbing Strain	Serum Strain
253	2,000+	50+	50+
M 254,1	2,000+	25+++	50+
217 L	1,000++	0	0
246 L	1,000++	0	25+
247 L	400+++	0	50+
248	1,000+++	25++	25++
250 L	1,000+++	25++	25++
251	2,000+	25++	25++
252 M	1,000+++	25++	25++

As is noted in table I, both type A and type B were isolated from case 254. This led us, when possible, to make numerous fishings from the plates inoculated with the sputum from the cases of pertussis which came to us later. These fishings have been tested and grouped either by agglutination alone or by agglutination and agglutinin-absorption. The number of cases and fishings per case thus tested are noted in table 5. All the cases with the exception of 254 have yielded only strains of group B.

The grouping of all the strains studied may be summarized thus: Among the old stock strains, both types were encountered. In some instances, transplants of the same cultures carried by different laboratories have been

TABLE 5.

TABULAR SUMMARY OF STRAINS FROM INDIVIDUAL CASES

Cases	Number of Fishings Tested	Grouping			
		Group A		Group B	
		Classified on the Basis of Direct Agglutination Only	Classified on the Results of Agglutination and Absorption	Classified on the Basis of Direct Agglutination Only	Classified on the Results of Agglutination and Absorption
M 254	4	0	1	0	3
M 255	20	0	0	14	6
M 256	21	0	0	16	5
P 257	2	0	0	0	2
P 258	15	0	0	11	4
P 259	7	0	0	4	3
P 260	4	0	0	2	2
P 261	2	0	0	1	1
P 262	35	0	0	26	9
P 263	15	0	0	11	4
P 264	1	0	0	0	1
P 265	1	0	0	0	1
P 266 a+	1	0	0	0	1
P 266 b+	1	0	0	0	1
P 267	3	0	0	2	1
P 268 a+	3	0	0	2	1
P 268 b+	3	0	0	2	1
P 269	3	0	0	3	0
P 270	3	0	0	3	0

a+ and b+ = successive isolations from the same case with an interval of two weeks.

found to be different. This must be due either to mislabeling or to the fact that the original strains contained a mixture of the types. In one instance there was apparently a mixture in one of the stock cultures. From the recent cases, one case yielded both types, but all the other cases have yielded only type B. What interpretation is to be made of these results it is impossible to state. It may be that the different types may vary in their prevalence at different times; or it may be suggested that only one of the types is a true B. pertussis. This cannot be disproved, but the presumption is against this conclusion in view of the morphologic and cultural similarity as well as the agglutinative relationship. It may be argued that the differences are due to a change of serologic characteristics among some of the stock strains. This argument cannot be considered in view of the isolation of both types from a recent case (254).

In view of the report of Bordet and Sleeswyk that they could influence the antigenic characters of B. pertussis by the use of different mediums, all our serologic tests were carried out with bacilli cultivated on one type of medium, namely, chocolate agar. Bordet and Sleeswyk reported that if they immunized a horse or a rabbit with bacilli grown on a blood containing medium, such as the Bordet-Gengou medium, that the resulting agglutinins were active against bacilli grown on the same medium, but less active against bacilli grown on plain agar. The agglutinins could be completely absorbed from such a serum by bacilli grown on the Bordet-Gengou medium but not by the same strain grown on plain agar. If a horse or a rabbit were injected with bacilli grown on plain agar, the results were reversed. If the strain grown on plain agar were again cultivated on Bordet-Gengou medium, its agglutinative characteristics became the same as the strain continuously grown on a blood medium.

These results have never been confirmed to our knowledge. Povitzky found that horse injected with two strains grown on Bordet-Gengou yielded a serum during the earlier period of immunization which agglutinated bacilli grown on the same medium but not those grown on chocolate agar. After a longer period of immunization no such differences were noted. Working with rabbit serums she found no agglutinative or absorptive differences in regard to the mode of cultivation of the bacilli used.

We have made comparative tests with serums obtained from rabbits injected with B. pertussis grown on chocolate agar. Antigens were prepared from the bacilli grown for from 5 to 20 generations on plain agar, chocolate agar, Bordet-Gengou medium containing about 25% of unheated blood, and a medium studied by Povitzky. This medium was a potato-glycerol-veal agar which contained about 35% of unheated blood.

Table 6 is representative of the results obtained. With the older stock strains, no appreciable differences in agglutinative or absorptive characteristics were noted. With the more recently isolated strains, which, however, could not be grown on plain agar, considerable differences were observed. The antigens grown on chocolate agar (heated blood) agglutinated well and showed a high absorptive capacity. Those from mediums containing unheated blood (either Bordet-Gengou or Povitzky) agglutinated poorly or in some instances not at all and had a low absorptive capacity. A transfer for even one generation to chocolate medium resulted in a complete return to the higher level of agglutinability and absorptive capacity.

A serum from a rabbit injected with a recently isolated strain grown on the Bordet-Gengou medium was tested in the same way. This serum gave much better agglutination with bacilli grown on the chocolate medium.

TABLE 6

THE INFLUENCE OF THE MEDIUM ON AGGLUTINATION AND AGGLUTININ-ABSORPTION; SERUM BORDET, FROM RABBIT IMMUNIZED WITH BACILLI GROWN ON CHOCOLATE AGAR

Agglutination Before Absorption			Agglutination After Absorption				
			Absorbed By		Tested With		
Strain	Grown On	Titer	Strain	Grown On	Strain	Grown On	Titer
Bordet	Chocolate agar	3,000++	Bordet	Chocolate agar	Bordet	Chocolate agar	0
						Povitzky	0
						Bordet-Gengou	0
						Agar	0
Bordet	Povitzky	3,000+	Bordet	Povitsky	Bordet	Chocolate agar	0
						Povitzky	0
						Bordet-Gengou	0
						Agar	0
Bordet	Bordet-Gengou	3,000++	Bordet	Bordet-Gengou	Bordet	Chocolate agar	0
						Povitzky	0
						Bordet-Gengou	0
						Agar	0
Bordet	Agar	3,000+	Bordet	Agar	Bordet	Chocolate agar	0
						Povitzky	0
						Bordet-Gengou	0
						Agar	0
P 264,4	Chocolate agar	3,000++	P 264,4	Chocolate agar	P 264,4	Chocolate agar	0
						Povitzky	800+
						Bordet-Gengou	800+
P 264,4	Povitzky	1,600+	P 264,4	Povitzky	P 264,4	Chocolate agar	400+
						Povitzky	400+
						Bordet-Gengou	800+
P 264,4	Bordet-Gengou	3,000+	P 264,4	Bordet-Gengou	P 264,4	Chocolate agar	100+
						Povitzky	800+
						Bordet-Gengou	800+

When the serum was absorbed by bacilli grown on the chocolate medium, the agglutinins for the chocolate-agar-grown bacilli were removed; when absorbed by bacilli grown on the Bordet-Gengou medium, these agglutinins were only reduced. In neither instance, however, was there an appreciable reduction in the "agglutination" of the Bordet-Gengou antigen. These results are given in table 7. The fact that the absorption was not followed by a reduction in

TABLE 7.

THE INFLUENCE OF THE MEDIUM ON AGGLUTINATION AND AGGLUTININ-ABSORPTION; SERUM M 255 FROM RABBIT IMMUNIZED WITH BACILLI GROWN ON BORDET-GENGOU MEDIUM.

Agglutination Before Absorption			Agglutination After Absorption				
			Absorbed By		Tested With		
Strain	Grown On	Titer	Strain	Grown On	Strain	Grown On	Titer
M 255,4	Chocolate agar	2,000++	M 255,4	Chocolate agar	M 255,4	Chocolate agar	0
						Bordet-Gengou	800+
M 255,4	Bordet-Gengou	1,000+	M 255,4	Bordet-Gengou	M 255,4	Chocolate agar	50++
						Bordet-Gengou	800+
P 270.3	Chocolate agar	2,000+	P 270,3	Chocolate agar	P 270,3	Chocolate agar	0
						Bordet-Gengou	400+
					M 255,4	Chocolate agar	0
						Bordet-Gengou	800+
P 270.3	Bordet-Gengou	800+	P 270,3	Bordet-Gengou	P 270,3	Chocolate agar	50++
						Bordet-Gengou	400+
					M 255,4	Chocolate agar	0
						Bordet-Gengou	1,000+

the degree of clumping of the Bordet-Gengou antigen, raised the question whether the flocculation with this type of antigen was due to specific agglutination.

The two types of antigens of the freshly isolated strain were tested, therefore, with 2 normal rabbit serums; also with 2 homologous serums, one from a rabbit injected with bacilli grown on the Bordet-Gengou medium, the other from a rabbit injected with bacilli grown on chocolate agar. Both the immune serums agglutinated the chocolate-agar antigens, whereas the normal serums did not. All the serums, including the normal, "agglutinated" the antigens prepared on Bordet-Gengou medium (horse blood) equally well. Antigens grown on a Bordet-Gengou medium containing rabbit blood were not "agglutinated" by the normal serums but showed a slight reaction with the immune serums. At first glance this would seem to indicate that the clumping of the Bordet-Gengou (horse blood) antigen was a precipitation reaction between rabbit and horse serum. Antigens of old stock strains, however, did not show the same results. As has been noted in the foregoing, the agglutinability and absorptive capacity of the older stock strains were not influenced by the medium on which they were grown. Why only the freshly isolated strains should be influenced by the medium and why only the antigens of such strains grown on unheated blood mediums should give nonspecific clumping, remains to be ascertained. These results again emphasize the dangers of employing antigens obtained from mediums containing unheated blood or serum. The recent report of Kelly, who found similarly bizarre reactions in studying the precipitin reaction in gonorrheal infections, is of special interest in this connection.

The results we have obtained do not in any way confirm the findings of Bordet. The observation of Povitzky that the antigens of recently isolated strains grown on the Bordet-Gengou medium were agglutinated much better during the earlier period of immunization is apparently explained by our observations; that is, the reaction was due to the horse serum without regard to its content of specific agglutinins.

Summary

A series of strains having the typical morphologic and cultural characteristics of B. pertussis have been studied by means of agglutination and agglutinin-absorption. These tests have demonstrated that the cultures studied fell into two serologic groups. These groups are designated as A and B. Antiserums for group B agglutinate the strains of group B but agglutinate the strains of group A slightly or not at all. Group A serums, however, agglutinate not only the strains of group A, but also agglutinate the strains of group B to a considerable extent. The absorption of group B serums by strains of group A does not appreciably reduce the content of agglutinins for strains of group B. The absorption, however, of group A serums by strains of group B results in a reduction in the agglutinins for strains of group A. The serologic differences are therefore sharply defined in one direction, but group relationship is shown in the reverse direction. The strains within each group are alike as far as is indicated by the absorptions of serums for different members within the same group.

The antigens for these tests were all prepared from the same medium; the serologic differences cannot be attributed therefore, to the influence of different mediums. The growth on blood mediums influences the ag-

glutinative characteristics of freshly isolated strains of B. pertussis but we have been unable to confirm Bordet's observations on this point.

The existence of two groups of B. pertussis is of immediate interest because of their possible bearing on the use of pertussis vaccine.

With the exception of one case of whooping cough which showed the presence of both types, all the recent cases have yielded only strains of group B.

For bibliography see the original article.

PERTUSSIS VACCINATION AS A PROPHYLACTIC MEASURE

Anna I. Von Sholly

During 1920-1921 the children under 10 years of age in 24 institutions were inoculated as follows:—1823 children received pertussis bacillus (hemophilus pertussis) type A saline solution, 3 strains varying from 8 days to 6 months old, dose 10 billion divided into 3 doses; 509 children received glycerole vaccine, pertussis A and B, 10 billion divided into 3 doses; 42 children received saline solution, type B. pertussis (original Bordet-Gengou bacillus). This made a total of 2374 children. At the same time 1971 children were inoculated with a vaccine composed of 6 strains of influenza bacilli, or with type 1, 2 and 3 pneumococcus vaccine—as controls.

Results:—Within the two following years whooping cough appeared in 11 institutions from two months to nineteen months after vaccination, as follows:—29 children who had been inoculated with type A pertussis vaccine developed pertussis, that is, 2.3% in the 11 institutions; 4 children who had been inoculated with glycerole A and B types of pertussis bacilli developed the disease, that is, 1.2% in the 11 institutions; 17 children who had been inoculated with pneumococcus or influenza vaccine developed pertussis, that is, 1.2% of the vaccinated control group in these 11 institutions. Among the unvaccinated controls, that is, children who were admitted subsequent to the vaccinations, 73 children or 6.7% of this group developed the disease.

In late 1922, two years after the first inoculations, the children of twelve of the same institutions were inoculated as follows:—of the children then in the institutions, those who previously had received pertussis vaccine were re-inoculated with the same, and those used as controls were continued as such. The new children were inoculated with pertussis vaccine and control vaccine alternately. Instead of type A pertussis vaccine, type B—the original Bordet-Gengou type—was used in 21 billion dosage divided into 4 doses. There were 605 re-inoculated children.

In all, 887 children received pertussis vaccine type B and 756 children received pneumococcus vaccine as controls, making a total of 1643 vaccinated children.

The results up to June 1st, 1924 are as follows:—whooping cough has appeared in 4 institutions among 4 children who received pertussis type B or 1.1% of this group in the 4 institutions; 9 children who received pneumococcus vaccine or 3.17% of this group of vaccinated controls; 39 children who were not vaccinated (new admissions) or 6.09% of this group.

In this investigation pertussis vaccine type B seems more favorable as a prophylactic agent than type A used in the first experiment.

POTENCY OF STORED PERTUSSIS VACCINES

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Lucy Mishulow, Carolyn Oldenbusch and Marie Scholl

The reports of the value of pertussis vaccine as a prophylactic and curative measure are contradictory. Some are favorable, whereas others indicate practically negative results. The claim has been advanced that freshly prepared pertussis vaccines are more antigenic than stored vaccines. Thus Huenikens reported his results on a series of children whom he immunized with pertussis vaccines. He treated one group with vaccines that were less than one week old; and a second group with vaccines that had been stored for varying periods. Two weeks after the last injection he tested their serums for complement-fixing antibodies. Thus measuring immunity by the stimulation of complement-fixing antibodies, he found that vaccines that had been stored for 2 to 3 months immunized in only about 12.5% of the number tested. On the other hand, freshly prepared vaccines employed within a few days of preparation immunized in about 94%. But the freshly prepared vaccines that he used and those that he designated as stored, were not the same preparations. He used commercial vaccines in his tests of stored vaccines and compared their potency with those that he prepared himself and used soon after preparation. In our experience with bacterial vaccines we find that there may be wide variations in the potency of different lots of vaccine prepared at different times using exactly the same technic. Therefore, in order to compare the potency of fresh and stored vaccines the same preparation should have been employed throughout the test. Others reported favorably on the use of fresh pertussis vaccines as compared with stored vaccines basing their observations on clinical results (Freeman, Moody and others).

The continuous preparation of fresh vaccines on a large scale is laborious and time consuming. For this reason it is undesirable unless proven to be of greater efficacy than properly stored vaccines. The investigations, therefore, that we carried out have been to determine primarily whether the antibody response of laboratory animals injected with freshly prepared and with stored vaccines would yield any data corroborative of the above claims. The following questions concerning the best method for preparing potent pertussis vaccines were also investigated. Does a freshly isolated strain have greater antigenic value than a strain that has been under artificial cultivation for a number of years? Do vaccines that are grown on chocolate agar differ in antigenic value from vaccines that are grown on Bordet-Gengou medium? Have salt solution preparations the same keeping qualities as glycerol preparations? Does heating the vaccine have any effect on the potency of stored vaccine? Have preservatives any effect on the potency of stored vaccine?

Several lots of vaccines were prepared and tested as follows.

Lot 1.—A strain of pertussis that has been under cultivation for a number of years was grown on coagulated blood agar (chocolate agar) for 48 hours then the growth was divided into two parts.

Vaccine 1: The growth was scraped into sterile 0.85% salt solution, shaken by hand to break up clumps, and heated at 53° C. for one hour. It was then tested for sterility and enough phenol added to make 0.5% of the bulk. The concentration was determined by Wright's method and the vaccine was diluted with 0.3% phenol in 0.85% saline to contain 1,000 million bacteria per cc.

Vaccine 2. The growth was scraped into sterile 66⅔% glycerol in salt solution and shaken in a mechanical shaker for one-half hour to break up clumps. It was standardized by Wright's technic and further diluted with 66⅔% glycerol to a concentration of 40,000 million bacteria per cc. Phenol was added to make 0.3% of the bulk. Just before using, the vaccine was diluted with sterile physiologic salt solution (which had 0.3% phenol) to contain 1,000 million bacterial per cc.

Lot 2.—Vaccines 3 and 4 were prepared in the same manner as lot 1, with salt solution and glycerol respectively, only a freshly isolated strain was used instead of the old stock strain. The object was to determine, if possible, whether the length of time of artificial cultivation of the strain has any influence on the antigenic value of the vaccine.

Lot 3.—In this preparation a mixture of three old stock strains was used, all of them serologically identical (type A). They were grown on Bordet-Gengou medium for 48 hours and the growth scraped into sterile distilled water. The clumps were broken up by shaking by hand and after the concentration was determined by Wright's method, the vaccine was divided into two parts; one part was heated at 53° C. for one hour. Then an equal volume of sterile 1.7% salt solution was added to both the heated and unheated vaccines thus making the concentration of NaCl in the vaccine 0.85%. Each vaccine was again divided into two parts and phenol was added to alternate lots as follows: vaccine 5, unheated, no phenol added; vaccine 6, unheated, 0.5% phenol; vaccine 7, heated, no phenol added, and vaccine 8, heated, 0.5% phenol.

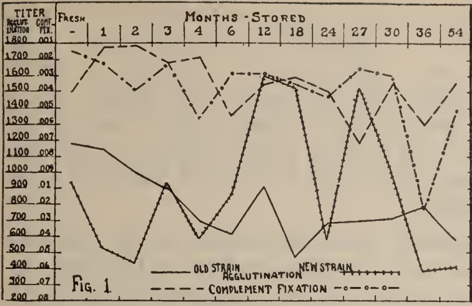
The object of this vaccine was to determine whether the medium on which the vaccine was grown and the heating of the vaccine in the process of preparation had any influence on the potency of the vaccine. Also, if there is any deterioration during the time of storage, whether phenol has any influence on the rate of deterioration.

Lot 1 (vaccines 1 and 2) and lot 2 (vaccines 3 and 4) were tested for potency as soon as they were found to be sterile, usually within two days, and the bulk was stored in the icebox at 8° C. to 10° C. and tested at intervals for four and one-half years (figs. 1 and 2). Due to pressure of other work at the time, vaccine lot 3 was not tested until after it was stored for 10 months, but after that it was tested at intervals until it was five and one-half years old (fig. 3).

Fresh vaccines were prepared of the strains used in the stored vaccines, and tested at the same time as the stored vaccines in order to control, more or less, the response of each lot of rabbits to immunization (figs. 4 and 5).

Method of Immunization.—A series of 4 to 6 rabbits was used for each of the preparations and each of the rabbits received 250 million bacteria intravenously for the first dose, followed by two more doses of 500 million each at intervals of 4 or 5 days. The rabbits were bled on the seventh day after the last injection and their serums were tested individually for agglutinins, and complement-fixing antibodies. Also the serum of each rabbit was tested before immunization to rule out nonspecific reactions.

Methods of Testing.—Agglutination: After some preliminary tests we found that antigens prepared of the old stock strain and of the freshly isolated strain gave practically the same titer of agglutination with



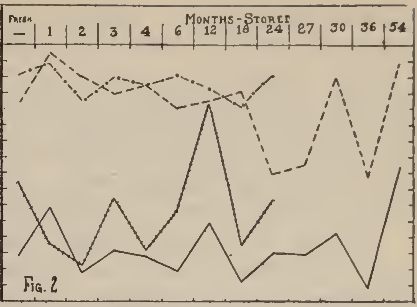


Fig. 1.—Complement-fixing and agglutinating antibody response of rabbits immunized with salt solution vaccines. Growth on chocolate agar; stored and tested at intervals.

Fig. 2.—Response to immunization with glycerol vaccine, similarly prepared and stored.

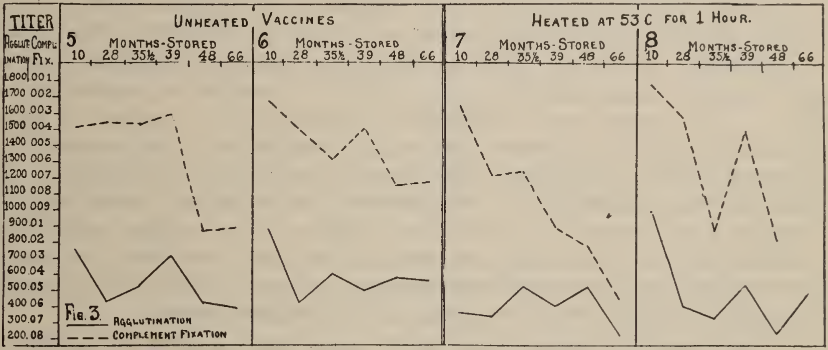
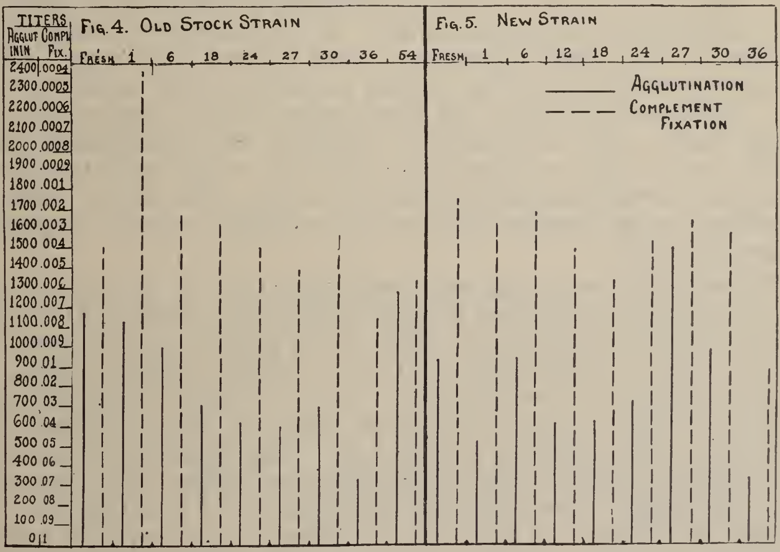


Fig. 3.—Response to immunization with salt solution vaccine. Growth on Bordet-Gengou medium; storage at 8°—10° C. Preparations 5 and 7 unheated and heated, respectively, contained no preservative, and 6 and 8 contained 0.3% phenol.



Figs. 4 and 5.—Variations in response of rabbits to immunization with freshly prepared salt solution vaccines. Growth on chocolate agar. These recorded responses were to freshly prepared vaccines, and were used as controls, respectively, for vaccines stored from 1 to 54 months as indicated by the figures of the heading.

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all the serums tested. Antigens of the old strain gave somewhat more uniform results and were therefore selected for testing all the preparations. A 24 to 48-hour growth on chocolate agar was scraped into a solution of formaldehyde (0.1% formalin in physiologic salt solution) and shaken for a few minutes to break up clumps. It was filtered through cotton and diluted with the formaldehyde-salt solution to the desired concentration, which was determined by preliminary tests. The antigen can be kept in the icebox for a year or more unless it is contaminated.

Serums of normal rabbits frequently gave agglutination in dilutions below 1:50; but in only one instance was there more than a trace of agglutination in a 1:50 dilution. Therefore to rule out nonspecific agglutination, the lowest dilution of the immune serums in our tests was 1:100. The serums were diluted 1:10, 1:20, etc. with physiologic salt solution. Then to 0.1 c.c. of each dilution of the serum, 0.9 c.c. of the antigen was added and the tests were incubated at 55° C. overnight. The highest dilution of the serum that showed strong agglutination was taken as the titer of that serum.

Complement Fixation: A 24 to 48-hour growth on chocolate agar was scraped into the formaldehyde solution (0.1% formalin in physiologic salt solution). The clumps were broken up by shaking and the suspension filtered through a thin layer of absorbent cotton and centrifuged. The sediment was resuspended in the formaldehyde-salt solution and again centrifugated. The sediment was then suspended in a formaldehyde-salt solution (formalin 0.5%) and shaken for 7 hours in a mechanical shaker. The antigen was titrated with rabbit immune serums and so standardized that four times the amount used in the tests was not anticomplementary and one-fourth of the test dose gave complete fixation with four units of the immune serum.

For the hemolytic amboceptor rabbits were immunized with washed red blood cells of sheep and their serums used as hemolytic amboceptor.

Guinea-pig serum was used for complement. Not all guinea-pig serums could be used in the tests as some gave nonspecific fixation and others gave weak or no fixation with positive serums. It was therefore necessary to test the serums of each guinea-pig before it could be used in the tests. Four to six guinea-pigs were bled on the morning preceding the day of the tests. As soon as the blood was clotted and some serum could be obtained by centrifugating, each serum was tested for hemolysis and complement-fixation. Those that were found suitable were pooled and stored until the following morning at a temperature slightly above freezing. The pooled serum was then titrated for the hemolytic unit, and was so diluted that 0.1 c.c. contained two hemolytic units.

The serums before testing were inactivated at 56° C. for ½ hour. Each serum of the rabbits immunized with the vaccines was diluted 1:10 and 1:100 with physiologic salt solution and titrations for antibody content were made, using two units of complement and 0.1 c.c. of the standard antigen. The tests were incubated in a waterbath at 37° C. for one hour to allow fixation to take place and then 0.2 c.c. of sensitized sheep red blood cells were added and the tests reincubated for another hour. The hemolytic system was so adjusted that the controls of the serum without the antigen, also of the antigen without the serums, were completely hemolysed in 15 to 20 minutes. The additional incubation of the tests after the controls were hemolysed helped to further rule out weak or doubtful reactions as the unhemolysed blood cells would settle to the bottom of the tubes leaving the supernatant fluid clear in the tubes where fixation was complete. In charting the results, the smallest amount of the serums that gave complete fixation (++++) was taken as the titer of that serum.

In testing the serums of the rabbits immunized with vaccines lots 1 and 3 for complement-fixing antibodies, an antigen prepared of the old strain was used. The serums of the rabbits immunized with vaccines of the new strain were tested with both the old strain and the new strain. There was very little difference in the titer of the serums when tested with the two antigens and for the sake of uniformity the results with antigen from the old strain were taken for all the different lots of vaccines.

The serum of each rabbit was tested for complement-fixing antibodies before immunization. In no instance did we get strong fixation in .01 c.c. of the serums and only very rarely was there strong fixation in .02 c.c. Usually they were either completely negative or gave only partial fixation in .02 c.c.

We found considerable variation in the individual response of rabbits in each test. In order to obtain a fair estimate of the results, although each rabbit was tested individually, we used in charting the average response of each series of rabbits in each test (figs. 1, 2 and 3).

DISCUSSION

The results of our investigations which extended over a period of five and one-half years and in which we tested several different lots of pertussis vaccines when freshly prepared and after storing, show that there was no measurable deterioration in their power of stimulating complement-fixing antibodies in rabbits. The vaccine of the old stock strain prepared in salt solution (vaccine 1) showed gradual slight deterioration of agglutinogenic power. But it is difficult to draw conclusions from that, as the salt solution vaccine of the newly isolated strain (vaccine 3) showed no such deterioration. The glycerol preparations of both the old stock strain and the newly isolated strain showed no deterioration in their power of stimulating agglutinins and complement-fixing antibodies in rabbits.

There was a marked fluctuation in the response of different lots of rabbits that were immunized with the various preparations as well as individual rabbits in each lot. But these fluctuations were similar in every respect to the fluctuations shown by rabbits when immunized with freshly prepared vaccines tested at different times (figs. 4 and 5). The fluctuations in the agglutinin response of rabbits to immunization with vaccine from the new strain were much greater than in those immunized with vaccine from the old strain; but on the whole they seem to be alike in their antigenic value.

The vaccines prepared of a mixture of three old stock strains and were grown on Bordet-Gengou medium, some of them heated and stored with and without preservatives were not tested until after they were 10 months old. Figure 3 shows that the unheated vaccine without preservative showed no deterioration until it was stored for 48 months. Then there was a decided drop in agglutinogenic power as well as in its power to stimulate complement-fixing antibodies. The same vaccine with phenol gave practically the same results. The heated vaccine without preservative showed a marked deterioration in its power to stimulate complement-fixing antibodies between 10 and 28 months storage, which continued decreasing during the entire period of testing (66 months) but showed practically no decrease in agglutinogenic power. The heated vaccine plus preservative, after an initial drop in both agglutinins and complement-fixing antibodies between 10 and 28 months storage showed no further deterioration, only the fluctuations due to the difference in response to immunization of different lots of rabbits.

The four preparations in this lot of vaccine (lot 3) were tested simultaneously; i. e., the rabbits were immunized simultaneously and their serums were tested on the same day with the same reagents. In this way the differences elicited can not be attributed to possible variations due to the reagents employed. The heated salt solution vaccine plus phenol was found to retain its potency much longer than the heated salt solution vaccine without phenol. The unheated salt solution vaccine with and without phenol retained its potency for a longer period than the heated salt solution vaccine without phenol; but not as long as the heated salt solution vaccine to which phenol was added.

Harvey and Iyengar in their experiments on pigeons found that agglutinin and complement-fixing antibody response run parallel with the protective power up to 10 months' storage. After that the protective value deteriorates, but the agglutinin and complement-fixing antigenic powers remain high for a much longer period. We could not investigate this claim, as the pertussis bacillus is not sufficiently virulent for laboratory animals to test the protective value of the vaccine. In regard to the production of agglutinin and complement-fixing antibody, however, our results may be summarized as follows:

Pertussis vaccines prepared in salt solution, heated at 53 C. for one hour, containing 0.3% phenol, were found to retain their power of stimulating the production of agglutinins and complement-fixing antibodies in rabbits for a period of from 4½ to 5½ years, when stored at 8° to 10° C. When stored without preservatives such heated, salt solution vaccines deteriorated more rapidly than either the heated vaccine plus preservative or the unheated vaccine. Unheated salt solution vaccine with and without preservatives differ very little from heated vaccines to which preservative was added, when tested after a comparatively short period of storage. On longer storage there was considerable deterioration.

Vaccines prepared of a newly isolated strain of pertussis showed no greater antigenic power than vaccines prepared of an old laboratory stock strain.

Glycerol preparations of both the old stock strain and the newly isolated strain showed no appreciable deterioration in their power of stimulating agglutinins and complement-fixing antibodies in rabbits for a period of 4½ years.

Vaccines grown on chocolate agar did not differ in their antigenic power from those that were grown on Bordet-Gengou medium.

Conclusions

If the antigenic value of pertussis vaccines can be measured by their power to stimulate specific antibodies, and if the response in human beings to immunization with the vaccines is similar to that in rabbits, properly stored vaccines remain antigenically potent for a considerable length of time. The claim that stored pertussis vaccines lose about 75% of their antigenic potency after storage for 2 to 3 months, has not been substantiated. Eight different preparations, stored at 8° to 10° C. and tested periodically, were highly potent at the end of four and one-half years.

For bibliography see the original article.

A NOTE ON THE CLAIM THAT AGGLUTININS ARE LIPOIDAL IN NATURE

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Charles Krumwiede and W. Carey Noble

The claim that agglutinins could be extracted from sera by the use of lipoidal solvents, was called to our attention by the address of Jobling on the relation of lipoids to immunity. This claim was made by Stuber, who believed he had demonstrated that he could extract the agglutinins from an immune serum with petroleum ether and that the addition of the extracted substances to normal serum would result in an agglutinating value practically equal to that of the unextracted immune serum. He claimed, also, that the serum of normal animals became strongly agglutinative after the animals had received intravenous injections of such extracted substances.

We have attempted to duplicate the results of Stuber without success. Experiments were carried out with serum as separated from the clot and with dried serum. Extraction was done with ethylic ether in some experiments and with petroleum ether in others.

Conclusions

There is nothing in the above experiments to indicate that agglutinins are lipoidal in character to the extent that they are soluble in lipoid solvents. In no case were we able to reduce the titer of extracted sera beyond a reduction which is explainable by the manipulation of the serum. In no case was there a transfer of agglutinins by the extracting solvent where we could not at the same time demonstrate a transfer of antigenic proteins as demonstrated by the use of the precipitin test with anti-horse serum. The methods employed were relatively simple. This was desirable in view of the destruction of the antibody by more complex methods. However, the methods used would suffice to demonstrate the solubility of agglutinins in the solvents used, if they were soluble in these menstrua.

For bibliography see the original article.

AGGLUTININ ABSORPTION

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Charles Krumwiede, Georgia Cooper and Dorothy J. Provost.

I. Introduction

The absorption of agglutinins was first observed by Gruber and Durham (1896) or at least they noticed that during agglutination, there was diminution in the amount of agglutinins—"gebunden," "zerzetzt" (Gruber). Bordet (1899) employing normal serum which possessed agglutinating properties, showed that the absorption was specific; the addition of the cholera vibrio resulted in the loss of the agglutinins for this bacterium but not those active against the typhoid bacillus and vice versa. Hahn and Trommsdorf (1900) proved that agglutinins were actually absorbed, not inactivated or destroyed. By treating the agglutinated bacteria with weak alkalis or acids, they were able to recover at least some of the agglutinins. Eisenberg and Nolk (1901-1902) investigated the quantitative factors of the phenomenon of agglutinin binding as well as the effect of chemical and physical agents upon the serum or antigen and the influence of such treatment upon absorption.

Gruber and Durham and especially those that followed them, appreciated clearly that serum agglutination was only relatively specific; that a serum would agglutinate not only its homologous bacterium but also related varieties or even unrelated varieties. These heterologous types were usually agglutinated in lower dilutions than was the homologous type. In exceptional instances, however, surprising and apparently paradoxical results were obtained. Thus, the serum from a case of enteric fever might agglutinate equally well two or even more of the bacilli of the typhoid-paratyphoid group. In some instances, a variety that was not the infecting type, would be agglutinated to the greater degree. Some observers suggested that such paradoxical results were due to overlooked secondary infections. This explanation, however, was not acceptable for some of the apparently contradictory results were obtained in cases in which careful and repeated bacteriological examination excluded this possibility.

To eliminate such difficulties, Castellani (1902) suggested the practical application of the experimental knowledge on agglutinin absorption which had been made available by previous observers. To demonstrate the practicability of such an application, he presented the absorption results obtained with serums of animals immunized with one or more bacterial types. He advanced the following criteria as the basis of such practical application:

1. A monovalent serum absorbed by its homologous culture loses its agglutinating capacity not only for the homologous culture but also for such heterologous strains as it may have agglutinated. Absorption by a heterologous strain results in the loss of agglutinins for this strain without the loss of the agglutinins for the homologous strain.
2. A bivalent serum when absorbed by one of its homologous cultures loses the agglutinins for this culture and not for the second and vice versa; when absorbed by both, all the agglutinins are removed.
3. These results can be utilized in the diagnosis of mixed infections; also in the identification of an organism.

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[1]We are indebted for assistance to Allen Johnson, Katherine Cox, Lucy Clark, Elizabeth Waterman and Hortense Lebenstein.

Although agglutinin-absorption has been the subject of numerous papers, it is surprising to find after the lapse of over twenty years how many misconceptions still persist; first, as to the validity of Castellani's conclusions; second, as to the practical application of what is valid; third, the method of application; fourth, the limitations of the method, and fifth, the deductions that may be drawn from the results of its application.

The difficulty lies primarily in the fact that the literature on the subject is large and frequently contradictory.

No attempt at an exhaustive survey of the literature has been made, hence no priority in observation is necessarily implied where no references are given.

In presenting the subject the application of the absorption method to the identification of bacterial types is considered first. The validity of Castellani's deductions as regards diagnostic application is left for a later section.

As a basis for further discussion we should restate Castellani's theorems, thus: the culture used for injection absorbs from the resulting immune serum all the agglutinins, the production of which was due to the specific antigenic stimulus of this bacterium. The corollary of this is that the agglutinins in a serum, not absorbable by the homologous culture are not due to the specfic stimulus of the antigenic matrix of this bacterium. Under appropriate conditions a culture which absorbs all the agglutinins stimulated by and absorbable with the serum culture, is similar to the serum culture. A culture which absorbs only a part or none of the agglutinins is different from the serum culture.

These theorems, as restated, are discussed in detail in the following sections. The division of the subject matter under the headings given, is artificial and some overlapping and repetition results. In spite of these drawbacks, some such subdivision is necessary if emphasis is to be placed upon the essential sources of error and misinterpretation.

In the consideration of the subject of agglutinin-absorption it must be kept in mind that agglutination is a two-phase phenomenon; first, combination of agglutinin and bacterium; second, flocculation through physical action.

Many important points are not directly available under the particular heading of agglutinin-absorption but are scattered in articles on the study of different bacterial groups and types. None of the books on immunity or serology more generally used nor any of the sections on agglutination in the larger systems serves as an adequate source of information. In most of the texts, agglutinin-absorption is described primarily in relation to the theoretical aspects of agglutination. Other writers make statements which though true in themselves are wholly misleading when read with their context.

The absence of any adequate review has suggested an attempt to summarize what we consider the essential facts and fallacies of the agglutinin-absorption method. We felt that this attempt was justified by the experience gained in an extensive application of the method during the last five years. This experience has covered many groups of bacteria such as the typhoid-paratyphoid group, meningococci, streptococci, pneumococci, B. influenzae, and B. pertussis; also a discussion of these results with other workers and the privilege of verifying the nature of unusual types of bacteria which have been submitted to us. A study of the more recent literature has been a further stimulus in this direction.

In undertaking such a summary we are basing the conclusions, as far as possible, on our own observations. Some of the examples utilized in the discussion have accumulated during our work on different bacterial groups. More recently, intensive work has been carried out with the one aim of eliciting further examples. The selected number of protocols which are presented later give little indication of the entire amount of work. Many of the examples given appear to us much more striking than similar ones which have been presented in the past. We appreciate that many of the points discussed have been raised by others either on the basis of actual observation or speculation.

II. The Significance of the Absorption of Common or Group Agglutinins

Summary: Direct agglutination may or may not be a reliable index as to the nature of a bacterium as this agglutination may be due to common agglutinins. The absorption of common agglutinins may be employed for tentative separation into groups, but such separation has no inherent validity because such grouping may vary with the number and quality of the serums employed. Individual cultures may be erroneously classified by such a method. The absorption of common agglutinins is not a criterion of likeness. The absorption of specific agglutinins must be used to determine agglutinative identity. Information concerning the specific absorptive capacity is a necessary criterion for conclusions as to the constancy or significance of fundamental agglutinative characteristics.

III. The Absorption of "Polyvalent" Serums

Summary: The absorption of a polyvalent serum may give tentative information of some value in the case of related cultures if the cultures used for immunization are also available for the test. To obtain information that will be of any considerable value, however, it becomes necessary to test all the cultures, including the cultures used for immunization, after each absorption. The value of the results will depend on how much the animal injected has responded with the production of specific agglutinins for each of the cultures injected. This is difficult to determine with any degree of certainty. These difficulties become proportionately greater, the larger the number of cultures used for immunization.

The use of a serum for absorption when one does not possess the cultures used in the production of this antiserum is open to the objection that one is working with unknown reagents.

In either case cited above the sources of error are essentially the same as those discussed under the absorption of common agglutinins.

If the method is applied with all possible safeguards the work becomes as great or greater than that involved in the preparation and use of monovalent serums to determine specific absorption capacities.

IV. The Absorbing Dose, the Time of Contact, Etc.

Summary: It is recommended that the minimal absorbing dose of the homologous bacterium be determined for each serum. One and one-half to three times this dose is generally a satisfactory amount to select for carrying out a series of absorptions to determine the likeness or unlikeness of other cultures. In the case of very close relationships or where a culture shows variations from the normal, comparison of the results with different sized doses or the use of a less than complete absorption dose, may be necessary.

V. Testing the Degree of Absorption

Summary: As a general rule, it is highly desirable to test an antiserum after absorption as low as 5 per cent. of the original serum titer (highest dilution showing good agglutination). In some instances it may be desirable to go below this level. To obtain an adequate picture of the results of absorption the absorbed serum should be tested in dilutions covering the whole active range of the unabsorbed serum.

Tests throughout the whole serum range are especially necessary where titration of the absorbing doses is used as an accessory means of interpretation. Another reason for testing the whole serum range is the occurrence of zone-phenomena (next section). Lesser degrees of absorption to 25 to 50 per cent. of the titer are proportionately less adequate. The absorption to 25 per cent. may suffice for broad grouping but it may fail to separate very closely related varieties. Conflicting results using this criterion may be due to variations in dosage which would not be noted in the absence of tests in the lower dilutions. The adequacy of any selected percentage will be more stringent if one selects as a criterion of serum titer the highest dilution showing complete agglutination. There are many difficulties in applying such a criterion. The use of only one dilution of the absorbed serum as a measure of absorption opens many dangers of misinterpretation and is wholly inadequate.

VI. The Prezone Phenomenon

Summary: The absorption of a serum by bacteria, homologous or heterologous, may exaggerate a pre-existing prezone or may introduce one. This is due apparently in large part to the products of autolysis and to some extent to media constituents. The use of sedimented bacteria resuspended in fresh saline is therefore preferable for the absorbing dose. The prezone, if exaggerated, may lead to difficulties or to actual error when it is overlooked. The occurrence of prezone phenomena is another reason for testing absorbed serum through a wide range of dilutions.

VII. The Reciprocal-Agglutinin Absorption Test

Summary: The fact that culture A absorbs the agglutinins from serum B is no criterion that culture B will likewise absorb the agglutinins from serum A. The method of testing the mutual absorptive capacities of two or more cultures using antiserums for each, is termed the reciprocal absorption test. The reciprocal test is the ultimate method available for determining agglutinogenic likeness or unlikeness. Agglutinogenic likeness as determined by reciprocal absorption is usually paralleled by close similarity in other characteristics. Exceptions, however, do occur, that is, cultures differing essentially in cultural and pathogenic characteristics may be nearly, if not completely, indistinguishable by the reciprocal test.

VIII. The "Receptor Analysis" Method, the Character of Flocculation, Etc.

Summary: We have no definite knowledge as to what underlies the specificity of serological reactions. The concept therefore that each and every agglutinative response or reaction is a criterion of the existence of separate components in a bacterium and similarly distinct agglutinins is purely hypothetical and a "receptor analysis" method based on such conceptions has probably no validity. The method is, however, of value as a means of simple presentation of empirical data to emphasize apparent relationships and to obtain suggestions as to further observations. In this regard, its value is greatest when based on reciprocal reactions obtained with antiserums and their homologous cultures as essential reagents. Additional observations on the character of clumping with the use of heated and unheated antigens and on group reactions may serve to yield additional suggestive data. Such additional modes of observation cannot be used as substitutes for the basic reagents antiserum and homologous culture. The "double receptor-analysis" method has no advantage over the regular absorption method when applied simply as a means of identification or differentiation. The method may, however, yield data which is of value in attempting to explain difficulties in differentiation of certain allied bacteria.

IX. PRELIMINARY SUMMARY: LIKENESS AND UNLIKENESS

Thus far we have considered the application of agglutination and agglutinin absorption as applied to so-called pure cultures. In this mode of application we think of a culture as consisting of only one variety of bacterium having certain specific agglutinogenic agglutinative and absorptive capacities which permit its identification or separation from cultures of other varieties. Not uncommonly therefore we speak of it as a bacterial type. In the usual sense, this implies that we think of a pure culture as an entity. Naturally this is not actually the case as a culture consists of many individuals of many generations. If we do speak of such a culture as a bacterial type we may be assuming either that each of the individuals has essentially the same specific serological qualities or that the type characteristics depend on the characteristics of the mass and not on the individuals.

Agglutination and agglutinin absorption as generally used for indentification or classification purposes are applied to cultures as though they were entities. It seems desirable therefore to summarize the facts thus far presented in terms of bacterial cultures before considering the question of the constituents of the culture, that is before discussing whether a culture is homogeneous or not from the serological standpoint.

First there are the reagents themselves. There would seem to be no need of discussing the reliability of the cultures used for study, their source, purity, etc. In the discussion thus far we have not considered variables; such as differences in agglutinability, absorptive and antigenic capacity, etc. among cultures of like varieties. These factors will be considered in succeeding sections. The summary that follows assumes, therefore, for purposes of discussion that the culture is a constant.

The antiserums to be utilized require some comment. One may be compelled in ordinary routine work on the commoner bacterial varieties to use "stock" antiserums obtained from other laboratories. Too frequently such antiserums are utilized in investigative work without regard to the species of animal from which they are obtained, without a knowledge of the "normal" antibody content and without the cultures employed for immunization to serve as a control. From the facts thus far emphasized this method is evidently far from desirable and in the ultimate analysis means that the work done with such antiserums is really with unknown reagents. In fact, examples have been given which show that the contingency is not remote that an investigator using antiserums in good faith may be unwittingly working with group agglutinins. The necessity of a knowledge of the species of animals employed may be emphasized by reference for instance to the broad agglutinin content which has been observed by Park and others in antidysentery serum obtained from horses. The preparation of antiserums by the worker with due regard to the species employed and with all necessary controls is therefore emphasized again.

On the whole the rabbit is the animal of choice for the production of agglutinating antiserums. Some have recommended other species, sheep for instance, as yielding a lower content of group agglutinins. Our own experience does not indicate that either sheep or goats yield better serums in this regard. As individual animals vary to a considerable extent it would require more extensive comparative work to reach a definite conclusion. On the whole moderately young animals seem more desirable than old animals. Storing of antiserums may be advantageous as the normal and common agglutinin content seems in some instances to fall rather quickly.

Even where one prepares one's own antiserum oddities may be encountered as we have noted. It may be necessary, therefore, to prepare another antiserum for control purposes wherever any peculiar results are observed. Should an antiserum show marked cross-agglutination, the degree of which seemingly is out of proportion to the probable degree of relationship of the heterologous bacteria agglutinated, it may be desirable to prepare other antiserums. Another antiserum may show very much less common action and thus eliminate the necessity of absorption tests which would be required with a more broadly acting antiserum. A point often lost sight of is the possibility of intercurrent spontaneous infection of the animals employed by a bacterium related to the one used for injection. We have encountered this several times. In one instance this resulted from accidental cross-infection from animals injected with paratyphoid cultures for virulence tests to animals used for serum production. That is the infection was caused by a bacterium virulent for rabbits but not a bacterium ordinarily occurring in them. That this contingency is overlooked is frequently seen in the literature where workers draw certain theoretical conclusions from results which may well have been due to an intercurrent infection of the rabbit used for injection.

That the titer of the antiserum is an important consideration should need little emphasis after the previous discussion. It should be as reasonably high as is consistent with a duration of immunization which will not unduly raise the group agglutinin content. The working range of an antityphoid serum of a titer of 1:800 is not as satisfactory as one of 1:8000. On the other hand unduly potent antiserums are not desirable as the absorbing doses must be inexpediently large.

The pooling of two or more antiserums for the same bacterium or for presumably similar bacteria has been commmented on and is to be condemned.

The use of the group-absorption method is only justifiable as a preliminary means of differentiation. No study of two bacteria by the group absorption method however extended will suffice to demonstrate their likeness.

The use of the polyvalent antiserums for investigative work is not to be recommended even where the homologous serum cultures are available. The use of polyvalent antiserums where the homologous serum cultures are not available has no justification. Their use resolves itself into a group absorption method with unknown reagents and its value is only that of the group absorption method.

If the group absorption method is employed for so-called "receptor analysis," it should be as a secondary and not a primary means. An apparent but not real exception will be noted later. The degree of absorption of the agglutinins and the standardization of the absorbing doses require attention. The minimal absorbing dose should be determined by actual trial. In general two to three times the minimum required when the homologous serum culture is used is a satisfactory absorbing dose to employ with other cultures, so that unimportant variables shall not confuse the results. The testing for the reduction of the agglutinin content to 5 per cent. of the original titer generally furnishes a safe criterion of agglutinogenic similarities or differences. Under certain circumstances titration of the absorbing doses and tests in dilutions lower than 5 per cent. of the original titer may be desirable.

Due attention must be paid to the introduction of phenomena caused by substances which accompany the absorbing dose and act as a "third colloid." The development of marked prezone reactions, the inhibition of spontaneous agglutination etc. have been discussed. Similarly disturbing reactions may be due to peculiarities in the serums.

Complete information as to likeness or unlikeness of two cultures is only obtainable by reciprocal tests with the cultures and their homologous antiserums. This means that a complete classification of a set of cultures is only possible where one prepares and tests antiserums for each of the cultures in the series. This is not always expedient and in many instances a reasonable certainty may be obtained with a less complete series of tests. This is not open to criticism unless it be presented or interpreted as complete and final evidence.

The reciprocal method of testing gives a reliable mode of determining the dominance and subsidiary position of the members of a group of bacteria that have very close relationship. Any attempt at an analysis of the presumable receptor or antigenic content or mosaic of a bacterium should be based primarily on such a method utilizing cultures and their homologous antiserums as the test reagents.

The necessity for reciprocal tests to determine complete identity indicates that in the last analysis the antigenic or antibody stimulating function of a bacterium is the essential one, that agglutination and agglutinin absorption are in fact only measures of this function. This conception implies that a bacterium is capable of absorbing all the agglutinins stimulated by it, and that the actual serum culture is the only control required for measurement. This conception must be considered fundamentally valid and absorption work primarily should be based on such a criterion even though exceptions may occur as will be noted later.

Usage has led to the application of the terms antigen, antigenic, etc., to the reacting reagent. Thus the bacterial suspension is referred to as the antigen, or a more extreme example is the use of the term "antigen" to designate the non-specific reagent in the Wassermann reaction. This has led to some confusion. Thus an observer on the basis of agglutination and agglutinin absorption using a few antiserums may conclude that he has demonstrated the existence of antigenic groups among cultures of a definite bacterial variety, as B. typhosus or B. paratyphosus B. Actually this is not so. As has been emphasized, an actual antigenic grouping would have to be based on the primary criterion, the antibody stimulating qualities of a bacterium.

A study of the character of the clumping of bacteria may yield data of theoretical and practical interest. Such observations do not add validity to the group absorption method. The character of agglutination may give presumptive evidence but does not serve as a complete substitute for the more certain agglutinin absorption results.

The fundamental requisite in agglutination and agglutinin absorption is therefore a culture and an homologous antiserum about which one possesses as full a knowledge as is obtainable.

X. VARIATIONS—CRITERIA

Summary: The ultimate criteria in the investigation of the occurrence of serological variations and of the kind or direction of such variation should be the study of the antigenic properties of the culture and its variants. This means the preparation of a homologous antiserum for each culture and for each strain or variant under study and a measurement of the antigenic response in each case by reciprocal agglutination and agglutinin absorption tests.

XI. VARIATION—PHYSICAL PECULIARITIES AND COLONY VARIANTS

Summary: Antigens from some cultures may show an instability which is reflected in their greater agglutinability or in actual spontaneous flocculation. Antigens from certain colony variants usually show this. If overlooked this may introduce difficulties in agglutinin-absorption or lead to errors in interpretation. Different colony types, rough, smooth, mucoid, etc., may be obtained from many bacterial types, the antigens of which differ in their characteristics. Such variants may give different results when their absorptive capacities are investigated. Where marked differences occur between the rough and smooth strains from a culture this is seemingly not a necessary concomitant of colony variation as similar differences can be elicited between two colonies of the same type. The rough strain, however, tends to show a generally lower absorptive capacity than does the smooth strain. The few mucoid types studied showed marked peculiarities especially in regard to lower agglutinability and low absorptive capacity. Only one "mucoid" antiserum was studied. The results indicated that the mucoid type differed antigenically from the rough and smooth types of the same strain. Whether this difference is an inherent characteristic of the mucoid type cannot be determined from one experiment. The failure to obtain differences with other types which uniformly paralleled the colony characteristics, indicates that the antigenic differences observed may be due to other factors than the mucoid characteristic alone. Some who have studied this phase have obtained more uniform agglutinative and absorptive differences between the colony types. It seems that when such marked differences occur other variation factors are associated. These variation factors which are indicated by antigenic changes, however, are not necessarily associated with variations in colony morphology.

XII. VARIATION—SEROLOGICAL HOMOGENEITY

Summary: Bacterial cultures may show a dual composition. The two components have been designated tentatively as "specific" and "group." This terminology is descriptive of the two components when they are studied in the group of closely related paratyphoid varieties. The essential agglutinative and absorptive differences between these related bacterial types depend on the specific component or fraction. The cross-agglutination and cross-absorption depend on the group component or fraction. This fact indicates a practical application namely, the use of the specific component or fraction in bacterial identification or classification or as an antigen in diagnosis agglutination work.

Certain limitations of this method as an ultimate substitute for absorption, are discussed. Even if pure component antiserums are not prepared a knowledge of the relative constitution of the cultures and antiserums is of great help in preventing false interpretations. The two components of any culture differ sharply from each other in regard to their reciprocal agglutinative and absorptive characteristics. Each component or fraction, however, possesses some of the characters of the other component or fraction; the group variety possesses those of the specific variety to the greater degree. This may be due to the pecularities noted in the breeding of these two components, the group variety showing a marked tendency to split off specific components, whereas the specific variety tends to breed pure. The dual constitution cannot be made to explain all group reactions. This duality of composition has been observed with the organisms of the paratyphoid group. Both components have been isolated from original material and from cultures. In some instances only one component has been found. Cultures yielding rough colonies have a duality similar to the smooth. Thus far a completely like condition has not been demonstrated for B. typhosus. The phenomenon of duality probably occurs in other bacterial groups, and should be considered and investigated in any classificatory study.

XIII. Variation—A Phenomenon of Degradation

Summary: Either stock cultures, strains from cultures or directly from infected persons may show evidences of loss of certain characters, which are characteristic of the bacterial race from which they originate. Such strains, therefore, have been termed "degraded variants." The degradation may vary in degree. The extreme degree among members of the typhoid-paratyphoid group is characterized by lessened or lost motility, a loss in agglutinability and an agglutination which occurs in fine clumps; also a marked loss in antigenic capacity. An antiserum homologous to such a degraded variant, may agglutinate the original culture in a higher dilution than the variants and absorption of such a serum by the degraded variant may fail to remove all the agglutinins active against the original culture. This is an exception to the general conclusion that a culture or strain will absorb all the agglutinins due to its specific stimulation. With antiserums for degraded variants of the typhoid-paratyphoid group, degradation may or may not be associated with a loss of specificity in that heterologous types are able to absorb the agglutinins from the degraded variant antiserum active against the degraded variant or even those active against the original culture. In some instances the degradation has resulted in an apparent increase in specificity. With B. proteus varieties it has been reported that an increase in specificity results from what is apparently a degradation process. Degradation is usually also followed by a loss in virulence. Colony variation may or may not be an associated phenomenon.[1] Although the phenomenon of degradation has been studied mainly with motile bacteria where changes in motility are associated with the change even if not a factor in it, similar degradation apparently occurs with non-motile species. The details of the changes which accompany degenerative changes among non-motile varieties require further study.

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[1] Since the above summary was written, reports by Arkwright (Jour. Path. and Bact., 1924, 24, 36) and Burnet (Jour. Exper. Path., 1924, 5, 251) have appeared. They use R (rough) and S (smooth) as synonymous with O and H the designations used by Weil and Felix for small and large clumping. We believe that they do not appreciate all the factors involved. Our results show that such a parallelism may occur, but it is not a necessary one.

The loss of specificity frequently shown by the degraded variant of the typhoid-paratyphoid group indicates that those characters which are associated with fine clumping underlie to some extent the phenomenon of group agglutination. Whether this factor in group agglutination is distinct from or related in some way to the group reactions due to group components cannot be definitely stated. The fine clumping factor is seemingly the specific element with some paratyphoid types. There is insufficient data to warrant a conclusion that in such instances the degraded strain originates from the specific component.

The failure of reciprocal absorption between a type and its degraded variant introduces a difficulty in interpretation, that is, whether one is dealing with actually different race types or with such a type and its degraded variant. When the culture shows other characters of degeneration the probability is in favor of the latter presumption. This is a distinct practical difficulty which may be encountered in routine examinations. If the degraded variant reverts to its race type, this will eliminate the difficulty.

Some degraded variants have shown only an apparent loss of certain race characters; these we have characterized as "vertical variants." Others have shown the apparent acquisition of characters not possessed by the race type; these we have termed "lateral variants."

XIV. Variation—In the Host or in Transfer from Host to Host

Summary: Certain definite types of serological variation may be accepted as occurring in the infected host. Most of the reports on variation in the host and in the transfer from host to host are only hypothetical and other hypotheses might be equally applicable. More carefully detailed serological observations, which will satisfy all the necessary criteria are needed, before any conclusions are justified as to the frequency, direction and degree of serological variation which bacteria undergo in the host or in the transfer from host to host and the limitations this places on conclusions drawn from the practical application of agglutination and agglutinin absorption.

XV. Variation—The Limitations It Imposes

In this summary we shall consider only those variations which, so far as we know from the evidence available, may occur under "normal" conditions, that is, variations which have been observed with strains obtained directly from the host or from cultures maintained by those methods, which presumably are adapted to an approximately natural mode of multiplication. It is the variations which occur under these conditions which are likely to lead to embarrassment in the practical application of the absorption method. As has been noted, very extreme variants apparently may be obtained by prolonged cultivation in broth without transfer and by other equally artificial means. Such variations have biological and some practical interest in the study of variations and its direction. Naturally such extreme variations may occur under normal conditions and this must, therefore, be kept in mind. At present, however, observations indicate that such extreme variation is comparatively infrequently encountered and consequently is not often a factor in the practical application of the absorption method.

We have emphasized again and again the postulate that a completely positive reciprocal agglutinin absorption test is the ultimate criterion of serological likeness or identity of two bacterial cultures or strains and that the absence of a completely positive reciprocal reaction is a definite criterion of unlikeness. This postulate is fundamentally valid, but its unreserved application would not serve the actual practical purposes for which the agglutinin absorption test is usually applied. Thus the data on variation show that if the postulate were unreservedly applied, we should be confronted with the necessity of assigning to different groups the variants, which are obtainable from a B. paratyphosus B. culture for instance. Such variants are actually serologically different and the observed differences may correlate with other differences; nevertheless the name B. paratyphosus B. applies to all, as they fall within the observed limits of this variety. At present there is no immediate purpose to be served by adding a varietal name to designate them.

If such definite types of B. paratyphosus are really races, it is obviously necessary to determine the potentialities for variation of other bacterial groups, if we are not to be led astray. Such information is relatively limited, and its acquistion may be of considerable aid in interpretation. Thus reciprocal differences may be noted and a number of cultures may fall, therefore, into dominant and subsidiary groups. The problem is, whether these findings are a result of inherent and constant race type differences or whether the subsidiary appearances are simply manifestations of the range of variability of a single race.

Apart from the question of serological classification as such, there is the danger of misinterpretation in more immediate practical work. Assume that in investigating an epidemic of paratyphoid fever, we isolate (as an extreme possibility) different variants from different individuals. Having a knowledge of the limits of type variability we should not be confused or fall into the error of ascribing the epidemic to two or more types. The isolation of variants having degraded character introduces a distinct difficulty in identification. The correlation of all the characters will usually give a reasonable certainty. Complete certainty can be obtained in some instances only if reversion to type fortunately occurs.[1]

In this connection the general application of the agglutinin absorption test as a criterion in epidemiological studies, requires further comment. Thus we showed during the last pandemic of influenza that the cultures of B. influenzae obtained from cases were in general different each from the other. We concluded that such heterogeneity could not exist if B. influenzae were the primary etiological agent of the pandemic. That is, the causative agent being transferred rapidly from case to case, if inferences may be drawn from previous experience, could not be conceived as varying to this extent. Do the observations on variation, here described, suggest that such a conclusion was not warranted? Assume that B. influenzae were a defined race and that its range of variability were far wider than any thus far observed. Is it possible that, even under these conditions, we should have observed practically no likeness, or at least no similarities which would have suggested at least the presence of variations such as we have described? Even the most radical proponents of variation in the host, such as Seligman, predicate serological stability at the height of an epidemic. As a matter of fact, there is little likelihood that variants from the type race norm would be in the majority. Those that were encountered could be recognized in many, if not most instances, as variants from the epidemic race type if appropriate means were employed. Although somewhat outside the present subject, it might be well to emphasize that the reverse of the above epidemiological argument is not necessarily conclusive. One and the same bacterial variety might be isolated from a series of cases and this variety might still not be the primary etiological agent.

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[1] We have just encountered a convalescent case of typhoid fever whose feces contain no typical B. typhosus but only what correlates in every way with the degraded variants described in the previous section.

A knowledge of the potentialities of bacterial variation is also evidently of importance in carrying out agglutination tests with patient's serum for diagnostic purposes. The possibilities of more clean cut differential results with the use of the specific component has been referred to. The possibilities of error in the employment of group or degraded variants are evident from preceding discussions.

It is obvious that the value of antiserums for routine identification purposes may be influenced likewise by the character of the cultures employed for injection and that the use of variant cultures may result in errors. These factors have a direct practical bearing on the production of vaccines and antiserums.* More detailed study of these factors in regard to the production of protective substances would be required, however, to determine all the limitations due to these factors.

In general criteria outlined in the preliminary summary (section IX) are invalidated in no way by the facts presented on variation. Variation only imposes certain limitations which must be observed, and agglutinin absorption results must be interpreted accordingly.

There is an apparent limitation in the opposite direction, in that at times the agglutinin absorption method is insufficiently delicate. Examples of this have been given; the failure to separate a presumed B. abortus culture from a presumed B. melitensis culture, likewise the close absorption relationships of B. typhosus, B. pullorum and B. sanguinarium. As has been noted this limitation has some relationship to the phenomena which operate in the loss of specificity through degradation.

XVI. MAY A CULTURE BE UNABLE TO ABSORB ALL THE AGGLUTININS FROM ITS HOMOLOGOUS ANTISERUM

Summary: We have restated one of Castellani's theorems thus: the agglutinins, the production of which was due to the specific stimulus of this bacterium.

This restatement is necessary as an antiserum may contain "normal" agglutinins without regard to the specific stimulus. Some apparent exceptions to this theorem are noted. Actual exceptions have been observed thus far only in the case of degenerate variants, which may be unable to absorb all the agglutinins whose production it stimulated.

XVII. TECHNIC

See original article.

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* Difficulties in obtaining an adequate antibody response in horses for the production of curative antiserum are encountered from time to time. We have recently encountered considerable trouble in preparing typhoid vaccine of sufficient agglutinogenic value to pass the standard requirements. It might be worthy of note that the serum of the rabbits injected with these defective vaccines also showed an almost complete lack of complement fixing antibodies. Such difficulties are referable to degradation in the stock cultures.

XVIII. AGGLUTININ-ABSORPTION APPLIED TO THE SERUM OF INFECTED PERSONS; DIFFERENTIAL DIAGNOSIS OF DISEASE

Summary: There is no other means of measuring the specific agglutinin content of a serum except by using the bacterial type which is known to have stimulated the production of the agglutinins in a serum even though this means may not always be adequate. There is no basic validity therefore in a procedure which assumes the reliability of determining the infecting bacterium or bacteria by testing the absorptive capacity of several cultures on the agglutinins present in the patient's serum for each of such cultures. Such a procedure does not take into account all the possible alternatives. The observation of the character of the clumping and the use of the specific component for test purposes may give strongly suggestive results. The probable alternatives in such methods are discussed.

Bibliography: See the original article.

A STUDY OF THE SPECIFICITY OF THE ABSORPTION OF ANTI-BACTERIAL PRECIPITINS

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Charles Krumwiede and Georgia M. Cooper

In planning some experimental work on the immunological identity or non-identity of certain bacteria, it was found that a suitable agglutination antigen, that is, a stable suspension of uniform turbidity, could not be prepared from the cultures under consideration. This difficulty as is well known occurs with some bacterial types. The question therefore arose whether under the circumstances we could employ the precipitin reaction. Should sharp differences be noted with this reaction, non-identity would be established. Should, however, the antigens of two or more cultures be found to give equal or nearly equal precipitation with an immune serum it would be necessary to resort to the antibody absorption method to determine the ultimate identity or non-identity of such strains: How far would such absorptions reveal specific differences?

This question was raised because of the well known tendency of the precipitin-precipitinogen complex to bind immune bodies of all types as well as complement; a phenomenon utilized by Gay, Chickering and by Weinstein to separate the antibodies of immune sera. The question of the specificity of such an absorption has apparently received little attention. Chickering found that an antigen of pneumococcus, type I or type II, removed only the immune substances for itself when a bivalent pneumococcus serum was employed. As these types are relatively very distinct and as they "cross-precipitate" to only a slight degree, these observations are of no significance as regards the question we have raised. Although we have not reviewed exhaustively the existing literature on bacterial precipitins, we do not believe that the fundamental question of the specificity of precipitin absorption has been studied with regard to bacteria which are closely related and therefore give similar or nearly identical reactions with precipitin sera.

We were in possession, fortunately, of cultures and serums which were especially suitable for a study of the above question. Smith and Ten Broeck called attention to the extreme degree of cross agglutination between *B. typhosus* and the avian types *B. sanguinarium* and *B. pullorum* when an immune serum for any one of these varieties was employed.

Extending these observations to the precipitin reaction we found that an anti-typhoid serum would precipitate the antigens of the above three types equally in the same dilutions and for the same periods. The similarity of precipitation was so close that the strains could not be differentiated one from the other by this reaction. We have also found that with an anti-pullorum serum similar cross reactions occur. Evidently, therefore, the serums and antigens of the three bacterial types mentioned above offer suitable reagents in testing the specificity of precipitin absorption. As a control, an antigen of *B. paratyphosus A* was used because antigens from this type were precipitated by the anti-typhoid serum in relatively low dilutions only. This type was therefore easily differentiated from *B. typhosus* by this reaction.

It was planned to carry out the absorptions in two ways; first, by utilizing the precipitin antigen; second, by utilizing the bacterial suspensions as is done in agglutinin absorptions.

The precipitin antigens[1] were prepared according to the method we have previously reported.

The technique is given in the original article.

The size of the absorbing dose in relation to the specificity or non-specificity of the results was given careful consideration.

Our inability to standardize adequately precipitin antigens may be a factor in the non-specificity shown with absorption by antigens which precipitate equally.

The absorption of precipitins from the immune serums by the use of precipitin antigens, or even by whole bacteria, was specific only within narrow limits. With a heterologous type which gave little precipitation, the absorption by the antigen of this type removed only the precipitins against itself. In the case of the heterologous types giving profuse precipitates, absorption by one of these types resulted in the complete removal of all the precipitins, even those active against the type homologous to the serum.

Assume in the case of the above cultures, that satisfactory agglutinating antigens were impossible of preparation, and reliance had to be placed upon the precipitin reaction. If we allow this assumption, it is obvious that the precipitin absorption would have given unsatisfactory results where its application would be required because of failure of differentiation due to cross-precipitation and would have given satisfactory results where it was not required because differentiation was evident on direct precipitation.

The results thus far recorded as regards non-specificity of absorption by the precipitin antigen itself were more or less expected. The result with absorption of precipitins by the bacterial bodies was so unexpected that it raised the question as to how much these sera cross-agglutinated and consequently what influence an equal absorbing mass of bacteria would have on the agglutinin content.

When the effect of a similar mass of bacterial on the agglutinin content of the serums was investigated, it was found that absorption of the serums by the homologous bacteria, using the same masses as in connection with the precipitin tests, reduced the specific titer only to a slight degree. Absorption by a heterologous strain, in the doses noted, also had relatively little influence on the height of the specific or group agglutinations. However, if the group titer was low, the heterologous strain might serve to remove the agglutinins for the absorbing type, but would leave the specific titer nearly unchanged.

The non-specific results obtained with precipitin absorption in the above experiments induced us to try other related varieties of bacteria and their antisera in the attempt to verify the results recorded in the original article.

The agglutinin-absorption technic leaves us a practical and relatively wide working range as regards dosage. The precipitin absorption method on the contrary, as the experiments indicate, has a narrow range and may fail to reveal distinct differences, especially where strains are closely related, even with scrupulous regard as to the size of the absorption dose.

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[1] The term antigen is used in the sense of a reagent. The ability of antibody stimulation is not implied.

CONCLUSIONS

A precipitin absorption method has only a limited application in the differentiation of closely related types of bacteria which, because of such relationship, show marked cross-precipitation. The tendency in such a case is toward non-specific results; that is, the bacterium, heterologous to the serum, in removing the precipitins active against itself may also remove the precipitins active against the homologous type. Where the cross-precipitation is less marked, more specific results tend to appear after precipitin absorption.

The results are the same whether a precipitin antigen or the bacteria themselves are used for absorption.

The results may be influenced by our lack of a suitable method for the standardization of precipitin antigens of bacterial origin. The conclusions as given, therefore, are stated in terms of this limitation.

The non-specific results obtained may be referable, in some degree, to the narrow working range of precipitating sera.

The inadequacy of the precipitin reaction as a primary or single method for the differentiation of related bacteria, which are serologically still unclassified, is again emphasized. While similar failures in differentiation may occur with the agglutination reaction, such failures may be easily corrected by resorting to the absorption method.

For bibliography see the original article.

PRACTICAL EXPERIENCE WITH THE RAMON FLOCCULATION TEST IN DIPHTHERIA.

Olga R. Povitzky.

This test was given a full trial in the early spring of 1924, and the results were encouraging[1]. Since September 1924 the Ramon test has been employed regularly for following up the progress of our horses immunized against diphtheria, and closely checked by guinea pig titrations. At first we used a toxin which contained 4 L + per one cc. of toxin. With this toxin the tests were made with from 2.5 to 1 cc. of toxin per tube depending on the strength of the sera tested. Later on, with a toxin of 11 L + per cc., we used exclusively 1 cc. per tube. The varying amounts of the tested serum were deposited first in the regular agglutination test-tubes and followed by 1 cc. per tube of the toxin (previously carefully titrated by guinea pig and Ramon tests). The contents of the tubes were well agitated and put in the water bath at 50° C. With the strong toxin the tests had to be watched closely as the reaction in the indicating tube occurred very promptly (from ten minutes to one hour). With the weaker toxin, however, the reactions were not as prompt and took from one to six hours and sometimes longer. The great essential for this test is therefore a strong, stable toxin titrated for its L + value. The stronger the toxin is, the prompter will the result be. The economy in money and time, resulting from the use of this method for estimating the values of antitoxin and L + toxins, is self-evident. There are, however, slightly over 15% discrepancies (up to 75 units) between the guinea pig and Ramon flocculation tests (as indicated by the table). This leaves out the one very irregular horse. If we include him the percentage is over 22%. Thus on 169 consecutive bleedings of 18 horses there were 36 discrepancies ranging between 50-100 units. In one horse it went up as high as 200 units. The other 6 horses showed discrepancies ranging not higher than 10-25 units (which can be disregarded). Discrepancies ranging above 75 units are met with in a little over 4% of bleedings (outside of horse 87). If we include him the discrepancies above 75 units would be in 7.6% of bleedings. Whether the high discrepancies which usually occur in the highly immune horses (above 400 units) is due to a higher protein content, which influences either of these two tests, is to be elucidated.

Out of 169 bleedings of a total of 18 horses, the Ramon flocculation test, in 7 instances, showed an excess of 75 units over the guinea-pig test,—in other words, 33% more units than shown by the guinea-pig test, out of the same 169 bleedings, the guinea-pig test, in 6 instances, showed excess of 75 units over the Ramon flocculation test—a discrepancy of 16-33 per cent.

Five of these instances were in the bleedings from the same horse. These results were obtained in the first year of application of the Ramon test. After employing the Ramon flocculation test for three years the Writer is more and more convinced that this test is a valuable, reliable agent for testing the progress of the horses under immunization. At present it replaces almost entirely the use of guinea-pigs.

During these three years our studies have shown that the flocculation and guinea pig tests do not present as many discrepancies as they appeared to in the beginning. Thus it was learned that the serums of horses retain

DISCREPANCIES BETWEEN GUINEA PIG AND RAMON FLOCCULATION TESTS ON 18 HORSES.

Horse No.	No. of Bleedings	Discrepancies				Differences in percentages of units of antitoxin in cases of discrepancies above 75 Units
		No. below 75 units	Units	No. showing Units above 75	% above 75 Units	
28	12	3	50–60	0		
33	13	3	50			
42	12	4	About 75	0		
43	12	4	50–100	2	Less than 17%	Twice 33% more for Flocculation Test.
45	12	3	75–100	2	Less than 17%	Twice 33% more for Flocculation Test.
46	5	Very Slight	10–25	0		
49	7	1	45	0		
62	13	3	50–100	1	Over 7.6%	Once 33% more for Flocculation Test.
66	8		20–30	0		
68	12	1	100	1	Over 8.3%	Once 33% more for Guinea Pig.
72	11	3	40–100	1	Over 9%	Once 33% more for Flocculation Test.
78	12	3	70	0		
80	6		10–25	0		
81	2		About 25	0		
84	8	2	50	0		
85	5		10–25	0		
87	12	8	50–200	6	50%	Once 33% more for Flocculation Test.
88	7		10–25	0		Once 33% more for Guinea Pig Test. Once 32.6% more for Guinea Pig Test. Twice 16% more for Guinea Pig Test. Once 23.8% more for Guinea Pig Test.
Total 18	169	38	22%	13	7.6%	all discrepancies up to 75 units.

Total percentage of discrepancies without irregular horse (87) over 15%.
Total percentage of discrepancies above 75 units over 7.5% in 169 Bleedings.
Total percentage of discrepancies above 75 units without irregular horse over 4%.

their characteristics as in the slowness or rapidity of their flocculation reaction. If for instance no flocculation is shown within 1 to 2 hours with a serum which previously had shown reactions within that time, it is probable that the proportion between the toxin and antitoxin is wrong and the test should be made over instead of waiting for a possible later reaction. Some plasmas which do not give reactions in the water bath at 50° C. show a reaction in the incubator (probably some coagulation of the plasma by heat). When the toxin is very strong and the reactions occur very rapidly (from 10-30 minutes) it is best to use a water bath at 37° C.

Toxoids which are made from strong toxins (at least 11 L +) show very good flocculation, while those made from weak toxins show no flocculation. This would show that the Ramon test is not only an indication of the neutral point between an M.L.D. toxin and antitoxin, but also of a saturation point of toxin with antitoxin.

In the Writer's experience it is possible to obtain a Ramon flocculation test with concentrated diphtheria sera, when no acid has been used in the process of concentration. It is not necessary in this case to mix the concentrated with fresh antitoxin serum (Bayne Jones' Method) for the Ramon Test. Our concentrated Diphtheria Sera contain two portions, the one called *B* is precipitated by ammonium sulphate and dialyzed in salt. This portion gives a good Ramon flocculation test. The second portion called *A* is further precipitated out by acetic acid. It is this portion which calls for a mixture with fresh antitoxic serum for the reaction.

Of especial value is the Ramon flocculation test in experimental work. An intensive study of the best means for preparations of strong toxins (which work is in progress now) is made comparatively easy because of this test. Numerous toxins are being prepared and the results determined the same day without the use of guinea-pigs.

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[1] Reference: Povitzky, Olga R., and Banzhaf, Edwin J., Diphtheria toxin-antitoxin titration by Ramon method for practical application. Proc. Soc. Exp. Biol. and Med., 1925, Vol. XXII, pp. 11-13.

SPECIFICITY OF RAMON FLOCCULATION TEST IN SCARLET FEVER

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Olga R. Povitzky

The first paper by the Writer on this subject was presented before the Soc. of Exper. Biol. & Med., May, 1925.[1] The work was continued, and special experiments with various controls established the efficacy of this test for the determination of the potency of scarlet fever toxins and antitoxins. Bleeding of horses injected with scarlet fever toxin were tested each month, and the results, when comparison was possible, usually agreed with those of intracutaneous neutralization tests in children. The curve of the progress of the immunization of the horses with scarlet fever with its gradual rise, fluctuations and drops could thus be studied as efficiently as in the case of horses with diphtheria. Control experiments for the specificity of this test with normal serum, broth, diphtheria toxins and antitoxins, also antibacterial streptococcus and pneumococcus serums were repeated, and the negative results obtained previously were confirmed. The most important control, however, was the 1 per cent. sheep's blood pneumococcus broth used for the growth of the scarlet fever toxin, and incubated at the same time as the toxin. This blood control gave reactions not only with scarlet fever antitoxins, but also with diphtheria antitoxins, antipneumococcus and antibacterial streptococcus serums. That these were pseudo-reactions, due to the sheep blood and fortunately not manifested in the presence of the scarlet fever toxin (due probably to the modification of the blood and change of the *p*H reaction by the growth of the scarlet fever *Streptococcus hemolyticus* in the broth) was proved by the following results: 1. The reactions with the control blood broth appeared in all tubes at the time when there was not even a trace of a reaction with the mixtures containing scarlet fever toxin-antitoxin in the early period of the immunization of the horse. 2. When the reaction between the toxin and antitoxin occurred, it showed only in the neutralization zone, for example, the tubes with a great excess or deficiency of serum remained clear, while the reactions with the control appeared in each tube, irrespective of the amount of serum. 3. The pseudoreactions usually occurred in the form of a film, which could be removed before shaking up.

The following points were found to be essential for the successful result of the test: 1. A toxin standardized for the number of toxic skin doses by the Dick test in children. 2. As a preservative, phenol, preferably the crystals, in 0.3 per cent. solution is used. The ingredients, toxin as well as antitoxin, should be perfectly clear for the test. If they are not clear after centrifugalization they are passed through sterile filter paper. 3. A constant amount of toxin and varying amounts of serum are used for the test. For weak serum the toxin is used in 0.5 c.c. and for stronger serum in amounts of 1 cc. per tube. It is preferable to use large amounts of weak serum, rather than dilute the toxin even slightly. This is true in principle of every

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[1] Ramon Flocculation Test for Determining Potency of Antiscarlatinal Toxin-Antitoxin. Proc. Soc. Exp. Biol. and Med., 1925, Vol. XXII, pp. 426-428.

Ramon test. 4. The optimum temperature for the scarlet fever test is 37° C.; anything above tis point was found to inhibit the reaction. 5. The first reactions to appear in the test tube were found to correspond to those in the intracutaneous neutralization tests in children. 6. The reactions usually appear in from twelve to eighteen hours. With some serums it may be twenty-four hours or even forty-eight hours, especially in case of concentrated serum. Here also, as in diphtheria, the serums of certain horses preserve their characteristics as to the slowness or rapidity of their flocculation reactions. If, for instance, no flocculation is shown within eighteen hours in a case of a serum which previously has shown reactions within that time, it is probable that the proportion between the toxin and antitoxin is wrong, and the test should be made over, instead of waiting for a possible later reaction.

The agglutinin content of four representative scarlet fever horses was studied, because the results may have some bearing on the nature of toxin-antitoxin flocculation. Horses A and C had 85 toxin inoculations, 52 injections of killed cultures and 4 Dochez treatments. Horses 140 and 141 had 26 and 45 inoculations by the Dochez method, respectively. Horse C, which had the lowest antitoxin content by the Ramon test, showed the highest agglutinin titer (1:3,200, higher not tried). Horse A showed agglutination in 1:400, though the antitoxin content by the flocculation test was higher than that of the previous horse. Horses 140 and 141, on the other hand, showed the highest antitoxin content by the Ramon test, and at the same time did not give any agglutination in any dilution with the same bacterial antigen. These results would seem to indicate that the antitoxin content of the serum as manifested by the Ramon test is not dependent on the bacterial content of the serum for the reaction, contrary to the view propounded by Brofenbrenner and Reichert in their work on "The Nature of the Toxin-Antitoxin Phenomenon." Whatever the nature of the Ramon flocculation may be, the immediate object is to obtain a reaction between toxin and antitoxin for the purpose of determining the potency of one or the other. Substitution of an atoxic filtrate for toxin or of an antibacterial antigen for antitoxin as done by Bronfenbrenner and Reichert to prove their contention, does not constitute the Ramon test; it merely produces the inevitable bacterial precipitation reactions. These reactions, though useful for the determination of antigen-antibody content, are of no value for the quantitative determination of either toxin or antitoxin. Failure to obtain results by the Ramon test may be attributable to improper technic, such as dilution of toxin, too small amounts in the test tube for a clear-cut reaction, unsuitable temperature and, last but not least, wrong proportions between toxin and antitoxin.

ANNOTATION: It is very essential to have a toxin of high potency. Because the scarlatinal toxins deteriorate rather rapidly, they should be used reasonably fresh. The deteriorated toxin may still be of use for intracutaneous tests in children, after it has lost its flocculating power. The sera also should be fresh when used.

STUDIES ON THE PARATYPHOID—ENTERITIDIS GROUP

VII. Enteric Infection ("Food Poisoning") Due to Tapioca Pudding Contaminated With B. Cholerae Suis (B. Suipestifer)

Published in The Journal of Medical Research, 1922, Vol. XLIII, pp. 53-59

Charles Krumwiede, Dorothy J. Provost and Georgia M. Cooper

On August 30th, 1921, there was delivered to us a sample of tapioca pudding and some uncooked tapioca. These samples were accompanied by a report indicating that the cooked pudding was the cause of "food poisoning" among the members of a family "O."

The history of this family outbreak was as follows:

The family consisted of: A. O., 20 years; J. O., 10 years (sister of A. O.); Mrs. O. J., 24 years (sister of A. O.); E. J., 3½ years (child of Mrs. O. J.); J. J., 5 weeks (child of Mrs. O. J.).

Inquiry indicated that some item of the evening meal of August 26th, 1921, 5.30 P. M. was the cause of the "poisoning." One item of the meal, see Table I, was tapioca pudding prepared from a package of tapioca, part of the contents of which package had been used previously with no ill effect. The pudding was started at 2 P. M. A quart of milk, grade B pasteurized, delivered that morning was opened and poured into a sauce pan and brought to a boil. To this was then added the washed tapioca. The mixture was then "boiled" in a double boiler for three-quarters of an hour. While still scalding hot, three eggs were beaten and mixed with the pudding. One of the eggs used was in the household, the other two were purchased at the time from a "chain" store together with the pork chops and frankfurters (see below) to be used for the evening meal. The pudding was then placed in an open dish till cool, then in the ice-box. It was characterized as "cold" when served. Whipped cream (cream, pasteurized, purchased that afternoon) was added when served.

The source of each of the food items was investigated and no evidence could be obtained that they were the cause of trouble in other families.

Mrs. J. maintained that all the materials were in good condition, also that she had sufficient ice in the ice-chest and that the ice-chest contained no remnants of food at the time. Food was usually purchased only as needed. The fact that only two eggs were purchased for the pudding (see above) corroborates this statement.

As noted above, the evening meal was eaten at 5.30 P. M. August 26th. The items partaken of by the different members of the family are shown in Table I.

TABLE 1.

FOOD ITEMS, THEIR CONSUMPTION

Name	Ill	Pork Chops, Fried	Frankfurters, Boiled and Fried	Potatoes, Mashed	Peas, Canned. Boiled with Fresh Carrots	Milk "B," Pasteurized	Tapioca Pudding
A. O.	Yes	Yes	No	Yes	Yes	Yes	2 oatmeal dishes
J. O.	No	Yes	Yes	Yes	Yes	Yes	None
Mrs. J.	Yes	Yes	Yes	Yes	Yes	Yes	1 dessert dish
E. J.	Yes	No	Yes	Yes	No	Yes	½ dessert dish
J. J.	Yes	(Wholly Breast Fed)					

Clinically the disease was characterized by vomiting, cramps, diarrhoea, fever and prostration. The incubation periods are given in Table II. One case (A. O.) died. An autopsy was performed by Medical Examiner Dr. Emanuel Martin. Congestion of all the internal organs was very marked. The congested intestinal tract showed no ulcerations, but the mesenteric lymph nodes were uniformly enlarged to the size of "navy" beans. Cultures from the spleen at autopsy yielded no growth. The cause of death was reported as undetermined, possibly "food poisoning." The organs were submitted for chemical analysis to Dr. Alexander O. Gettler, whose examination was negative.

As was noted in Table I, one of those partaking of the evening meal ate none of the tapioca pudding and she was not ill. The data as brought together in Table II strengthened the assumption that the tapioca was at fault, in spite of the fact that the infant did not eat it.

TABLE 2.

AMOUNT OF TAPIOCA PUDDING IN RELATION TO THE PERIOD OF INCUBATION AND OUTCOME OF THE DISEASE

Name	Amount of Pudding Eaten	Approximate Incubation Period	Outcome
A. O.	2 oatmeal dishes	6-9 hours	Died
Mrs. J.	1 dessert dish	20 hours	Recovery
E. J.	½ dessert dish	20 hours	"
J. J. (baby)	None	?*	"

* Illness began about twenty-four hours after onset of mother's illness.

Paratyphoid-like strains were isolated from the tapioca pudding and from the liver of A. O. These were tested with the agglutinuating sera specific for the various paratyphoid types. A few of these sera agglutinated the strains to some extent. Two hog cholera serums, however (Hog cholera Arkansas from Schütze, Lister Institute, Hog cholera isolated from hog by Ten Broeck 1919, obtained from Theobald Smith), agglutinated all the strains to the titre of the serum (titre 1:6400+). The indication on direct agglutinuation was therefore in favor of B. cholerae suis (suipestifer).

Feces from three cases were examined after convalescence but B. suipestifer was not found.

A strain from the tapioca pudding and one from the liver were selected to immunize rabbits for the production of agglutinating serums. The agglutination and the agglutinin-absorption tests with these antiserums show that the tapioca strains and the liver strains are antigenically identical and that these strains are likewise identical with the hog cholera strain employed as the type.

Discussion.—The fact that one and the same type of B. cholera suis or B. suipestifer was isolated from the tapioca pudding, and from the liver of the fatal case, is seemingly conclusive evidence that the illness was due to this bacterium. That this bacterium was not present in a living condition in the other foods as served seems shown by the fact that J. O., who ate of every other food item except the tapioca pudding, was not ill. That the symptoms were not due to "food poisoning" in the sense that there was considerable preformed toxin in the tapioca pudding is indicated by the following:—At the most, the bacteria had only about two hours in which to multiply; furthermore, when we received the pudding it was not very toxic for experimental animals even when injected intraperitoneally.

Considering the fact that the number of B. cholera suis or suipestifer organisms in the pudding was not great, it seems more likely that the symptoms were essentially due to an intestinal infection. If this is so, the infant was probably infected by the mother. This view seems justified in spite of the fact that the infant's blood gave no agglutination in dilutions of 1:20. That the illness of the child was only a coincidence cannot be excluded, but the development of a sudden gastro-intestinal upset in a breast infant, who had been doing well, seems a less likely alternative.

How the tapioca pudding was infected is a question. The previous use of the dry tapioca and the negative bacteriological examination is against the assumption that the tapioca was contaminated. Even if it were contaminated, the subsequent heating should have sufficed to kill the contamination. The milk used in the pudding is excluded as it was pasteurized and then boiled. The eggs would be a very unlikely source and furthermore the pudding was scalding hot, when they were added. The cream was pasteurized and is excluded by the fact that the bacterium was found in the pudding to which no cream was added.

Two pork products were purchased with the eggs used for the pudding. That Mrs. O. J. contaminated her hands in opening the packages and thus transfer the bacterium from one or the other of the pork products to the pudding is, at least, a reasonable assumption. That the frankfurters were not the source seems indicated by the methods of manufacture. However, contamination of the outside of the casings, after the heating, cannot be excluded. That either this occurred, or that the pork chops were infected, is apparently the alternative forced upon us to account for the contamination of the pudding. If either the frankfurters or chops were contaminated, the subsequent cooking rendered them innocuous, for the one member of the family who escaped infection ate of both these items.

The conclusions seem warranted:—That the tapioca pudding was infected with B. cholerae suis from pork products. That a limited amount of growth took place, so that each of those eating the pudding became ill. That the illness was due to an infection and not an intoxication, the infection giving rise to symptoms in one case, in from 6 to 9 hours. The last conclusion is not in accord with the view that the acute symptoms, developing after a short incubation, are primarily due to toxin preformed in the food.

We are indebted to Mr. W. B. Drennan and to Inspectors Samuel Goldman and Samuel Zusman of the Bureau of Food and Drugs for information and coöperation in obtaining the specimens for bacteriological examination.

References

No references to the general subject of "food poisoning" seem necessary in this report. We have been able to find only one reference where tapioca pudding was apparently involved in a "food poisoning" outbreak. Sewall, Smith and Priestly (Jour. Roy. Army Med. Corps, 1920, 34, 510) report an extensive outbreak in a hospital. The authors consider the source of the infecting organism to have been the canned milk used in the pudding. Tea, to which this milk was added, was apparently a contributing agent in the outbreak. The strains isolated from some of the cases fell into the general group of "food poisoning" paratyphoid types. The agglutination and agglutinin absorptions were not sufficiently complete to definitely identify the type.

For cultural and serological separation and serological identification see:
Krumwiede, Kohn, and Valentine. Jour. Med. Res., 1918, 38, 89.
Krumwiede, Valentine, and Kohn. Jour. Med. Res., 1919, 39, 449.
Schütze. The Lancet, 1920, 1, 93.
Ten Broeck. Jour. Exp. Med., 1920, 32, 33.

STUDIES ON THE PARATYPHOID-ENTERITIDIS GROUP.

An Epidemic of Food Infection Due to a Paratyphoid Bacillus of Rodent Origin.[1]

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By Ole Salthe and Charles Krumwiede

On April 9, 1923, reports were made to the Bureau of Foods and Drugs that a number of persons were ill of "food poisoning." The reports were accompanied by a statement that this illness was due to the consumption of cream-filled crumb cake and eclairs. An investigation was immediately undertaken. This quickly showed that the food items mentioned were probably those at fault. They were the only common foods among the cases in the various families affected, and in each instance the purchases were made from the same bakery on the same date, April 5, 1923.

An investigation at the bakery showed that no stock had been carried from the previous day; that is, the illnesses in 53 cases brought together were associated with the baking of April 5th. Subsequently, six more cases of illness developed, as shown in Table 1. This table gives a summary of the cases, the items eaten, the baking involved and the approximate incubation periods, as indicated by the data obtainable.

The illnesses were characterized by the usual symptoms, nausea, vomiting, cramps, diarrhoea, some fever, headache and prostration. The severity was roughly proportionate to the amount of the infected food eaten. None of the infections was very severe and there were no deaths.

The bakery involved was a high class shop, clean, orderly and free of flies and vermin. The baking was done in the basement, which in point of cleanliness was above the average. No vermin was noted. The owner had three cats to prevent rodent invasion of the basement, which, according to his statement, was troublesome, if cats were not kept.

The personnel employed was cleanly, and none gave any history which could be interpreted as bearing on the outbreak. Several successive samples of feces, urine and blood were obtained from each individual in the bake shop and bakery; all were negative.

Our suspicion was immediately centered on the cream filling, because this would serve as a very good culture medium, if contaminated. The baked dough of the eclairs and crumb cake did not seem a likely source, for each individual piece would have had to be contaminated in the handling, subsequent to baking.

The cream filling was prepared by adding dried milk and sugar to water. This was then boiled. A mixture of cornstarch, eggs and water was then stirred into the above, the boiling being continued until a thorough mixture was obtained. The mixture was then poured into a wooden pail, and this

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[1] From the Bureau of Foods and Drugs, and the Bureau of Laboratories, Department of Health, New York City.

We are indebted to Inspectors E. M. Hoctor, Christopher Matthews, E. Bocker, J. Turner and J. Goechler, who collected the field data and obtained the materials for examination.

Miss Georgia M. Cooper, Miss Dorothy Provost, and Miss Marie Luckenbacker made the bacteriological examinations and carried out the serological work for identification.

TABLE 1
SUMMARY OF CASES

Number of persons ill per family:	Food consumed:	Baking of†	Eaten	Illness began:
4.....	Eclairs	April 5, 1923	April 5, P.M.	April 6. P.M. to April 7, A.M.
2.....	"	" " "	" " "	April 6, A.M.
3.....	Crumb cake	" " "	" " "	April 6, early A.M.
4.....	" "	" " "	" " "	April 6, noon to April 7, A.M.
3.....	Eclairs	" " "	" " "	April 6, noon.
2.....	Crumb cake	" " "	" " "	April 6.
1.....	" "	" " "	" " —	April 6.
2.....	" "	" " "	" " —	April 6.
2.....	Eclairs	" " "	" " —	April 5, P.M.
1.....	"	" " "	" " —	April 5, P.M.
2.....	"	" " "	" " P.M.	April 5, P.M. to April 6, A.M.
1.....	"	" " "	" " "	April 6, A.M.
5.....	"	" " "	" " "	April 6, A.M.
4.....	Crumb cake	" " "	" 6 —	April 7, noon.
1.....	" "	" " "	" 5 P.M.	April 6, A.M.
2.....	" "	" " "	" " "	April 6, A.M.
1.....	" "	" " "	" " "	April 6, A.M.
4.....	" "	" " "	" " —	April 5, night.
3.....	Eclairs	" " "	" " —	April 6. A.M. to P.M.
4.....	"	" " "	" " —	April 6, A.M. to April 7.
2.....	?*	?*	?*	?*
3.....	Crumb Cake	April 9, 1923	April 9 —	April 11, A.M.
3.....	Eclairs	" " "	" 10 P.M.	April 10, night

* Home closed at time of inspector's visit; cases most probably connected with the April 5th baking.

†The pastry was prepared in early morning hours of the day noted.

stood on the floor, near the rear basement door. The mixture was usually stirred at intervals to accelerate cooling. Whether such stirring was actually done on the day in question could not be determined. The filling thus prepared was used for 120 eclairs, 50 cream puffs and 12 cream-filled crumb cakes.

Although the heating in the preparation of the cornstarch mixture would exclude the raw materials actually used, there remained the possibility that contaminated raw materials might find their way into the mixture after cooling. All open packages of raw materials were, therefore, embargoed and examined bacteriologically. All gave negative results.

The inspector, visiting each of the household involved, succeeded in obtaining left-over samples of both cake and eclairs of the April 5th baking. Bacteriological examination showed the presence of bacilli of the paratyphoid group. Because of other work which we were carrying out on the serological relationship of members of this group, we were able to make an immediate tentative identification of the bacilli present as *B. pestis caviae*. This identification was subsequently verified by a complete serological study of not only the agglutinative, but also the antigenic and absorptive characteristics. Stool specimens from 11 of the patients yielded, in 6 instances, bacilli of the same variety; they were shown to be like those in the cream in regard to their agglutinative, antigenic and agglutinin absorption characteristics. The details of the serological tests are not given here, but will be included in a later study on the serological classification of the paratyphoid group.

The results of the bacteriological examinations of the feces are tabulated (Table 2) since they have a practical value as indicating to some extent the persistence of the infecting type in the intestinal tract.

TABLE 2.

FECAL EXAMINATIONS

Case	Onset	Feces collected	Results as to presence of *B. pestis caviae*
D. P....	April 5th—night	April 11th	Positive
V. H....	April 5th—night	April 11th	Negative
S. M....	April 5th—night	April 11th	Positive
Mrs. F..	April 5th—night	April 11th	Positive
M. F....	April 5th—night	April 11th	Positive
M. J....	April 10th—night	April 17th	Negative
E. J.....	April 10th—night	April 17th	Negative
E. J.....	April 10th—A.M.	April 17th	Negative
H. B....	April 11th—A.M.	April 14th	Positive
F. J.....	April 11th—A.M.	April 14th	Negative
A. J.....	April 11th—A.M.	April 14th	Positive

The identification of the offending bacterium as *B. pestis caviae* gave us a definite clue on which to base a search for the source of contamination. Three possibilities immediately arose (see below, as to sources of *B. pestis caviae* type): bacterial rat viruses, rodents and the cats. The latter animals were removed to the laboratory and the feces (cage sweepings) examined seven times with negative results. No rat viruses had been used in the bakery or in any of the surrounding buildings. Traps were set and 11 mice, but no rats, were caught. Bacteriological examinations of the liver, spleen and intestinal contents of the mice were negative.

Before the above examination had progressed very far, a significant finding was made which apparently explained the mode of contamination of the filler. Besides a cleaning up of all utensils, etc., we insisted on the moving of all bulk supplies, and the shelves and floor were carefully inspected for the presence of rodent droppings. Rodent excreta were found on two boards used as storage shelves. Bacteriological examination showed that the excreta from the lower shelf were negative, but those from the upper one contained *B. pestis caviae.* This shelf was nearly over the place where the pail of filler was placed to cool.

How the rodent droppings got into the pail of filler is a question. Most probably this occurred through the agency of the cats jumping to the shelf in the endeavor to get at the mice or rats and thus scattered the feces into the pail.

The development of six subsequent cases, due to the baking of April 10th, was noted above (Table 1). An eclair was obtained from one of the families involved and stool specimens were also collected (Table 2). *B. pestis caviae* was isolated from the eclair and from two of the stools. This recurrence was evidently due to the same factors that gave rise to the first 53 cases.

The fact that only six cases developed from the baking of the 9th indicates that the contamination was slight and localized. This emphasizes a condition probably similar in regard to the baking of April 5th. As was noted in Table 1, 53 individuals in 21 families were made ill by the pastry of this day's baking. The total baking of this day consisted of 120 eclairs, 50 cream puffs and 12 crumb cakes. Ten of the families had eaten crumb cakes. This accounts, therefore, for at least 10 of the 12 crumb cakes prepared. Thirty-one persons ate eclairs. Even if a majority ate more than one apiece, over half of the number prepared is unaccounted for. No reports of illness due to the cream puffs were obtained. These facts indicate that the contamination of the crumb cakes was nearly uniform, that of the eclairs irregularly scattered, and that the puffs were either not contaminated or only to a negligible degree.

In the families visited, none who ate of the cream-filled cake or eclairs escaped at least some illness. It is difficult to understand how the irregularly contaminated eclairs happened by chance in the sales to go to the families in this way. This is especially true, as we know of instances, and there must have been many others, where eclairs were eaten without ill effect.

The method of filling the pastry may explain the peculiar distribution of the contamination.

The contamination must have been on the surface of the cream mixture, as it was cooling. If stirred this would tend to spread the contaminant. The following facts indicate that such stirring only served to spread the contamination in the upper layers. When cooled the mixture was poured into a filler, from which the eclairs were filled. Presumably the upper contaminated portion went into some of the eclairs which were filled first. The subsequent pourings into the filler were probably not contaminated. This would explain the absence of illness due to some of the eclairs and the cream puffs, which were filled after the eclairs.

The crumb cakes were the last item prepared. The cream was spread with a spatulum. Most probably the contaminated cream adherent to the side of the pail was scraped off, mixed with the remaining cream mixture in the bottom of the pail and then spread on the cakes. This procedure would explain the reason why nearly all of the crumb cakes were reported to have caused illness.

Undoubtedly multiplication of the contaminating bacteria played a considerable rôle in raising the infectiousness of the individual pastry items. As we pointed out in a recent report, this must be considered as a probably important factor, even where the time is short between preparation and ingestion. In the present epidemic a considerable time intervened in most of the cases (Table 1).

We have designated the causative strain as *B. pestis caviae,* because, among other reasons, in a previous study we had utilized a guinea pig strain as a type strain. In this work we were able to show by agglutination and agglutinin absorption that many of the paratyphoid strains, prevalent in rodent infections, were serologically alike and differed distinctly from the other paratyphoid types which had been differentiated up to that time. Some of the so-called strains of *B. typhi murium* from mice were found to be like *B. pestis caviae*. The majority, but not all the strains (exclusive of *B. enteritidis*) of guinea pig or mouse origin, were of this type. A strain from a rabbit and also one from a cat were likewise found to be of this type. The last was the reason for our attention to the cats in the bakery (see above). Since our earlier work, we have identified, as of this type, strains from hogs, a calf, a cow, a swell as strains from several avian species, also several strains from human sources. These will be discussed later. This would indicate that, if the type is primarily a rodent pathogen, it possesses a definite and well-marked ability to cause disease in many other species.

Schütze, in his serological study of paratyphoid strains, has added considerable data in this regard. Among the cultures studied, he separated a group which he designated as the "mutton type." Seven food infection strains, a strain obtained from one of sporadic gastro-enteritis cases, a strain obtained from a guinea pig epidemic, a strain from an epidemic among calves, one laboratory strain labelled psittacosis, presumably of avian origin, and other laboratory strains of unknown origin, fell into this group.

Through the courtesy of Dr. Schütze we obtained his "mutton" strain and it is identical with the strains we have designated as *B. pestis caviae*.

Undoubtedly, if one were to review carefully the serological data available in the various epidemics traced to paratyphoid types (exclusive of *B. enteritidis*), one could develop a presumption that a considerable number were due to the *B. pestis caviae* type, here described. Uhlenhuth and Hübener in their description of *B. typhi morium* cite a series of food infections which presumably were associated with the use of "rodent viruses." Were these viruses at fault and were it definitely shown that *B. enteritidis* was not contained in the viruses, there would be at least a presumption that the infections were due to the *B. pestis caviae* type.

As there is no certainty involved in these outbreaks, no summary of them is made. Now, that the prevalence of a definite serological type is recognized and available for comparative study, the relative incidence of *B. pestis caviae* can be established with certainty in future outbreaks.

As far as we know, the following is all the information available as to the incidence of this type in human infections on this continent. We have a culture of this type which according to the data accompanying it was isolated by Weinzirl in connection with food infection due to milk. We have been unable to find any published data, and do not know in which state this outbreak occurred.

The outbreak of food infection reported by Bernstein and Fish was caused by this paratyphoid type. Pie was the vehicle, and the culture was obtained from a cocoanut-custard mixture. We have the culture from this epidemic and have identified it as *B. pestis caviae*.

Winslow, Hiscock, Rogers and Robinson have recently reported an epidemic of food infection due to an egg salad. The contamination was seemingly of the mayonnaise dressing. They isolated the offending bacterium from the stools of some of the cases, and, on the basis of the cultural agglutination and agglutinin absorption reactions, termed the bacillus *B. paratyphosus B*. The agglutinations were carried out with an antiserum for a case strain and one for *B. paratyphosus B.* (Schottmüller).

Through the kindness of Dr. Winslow we have been able to add some of these strains to our collection and to test them with our various type antiserums. Our tests leave no doubt that the bacterium is a *B. pestis caviae* type and not a *B. paratyphosus B*. That is, it is different from Schottmüller type, although generically it might be considered a member of a *B. paratyphosus B.* group. It would take far too much space to go into the technicalities of agglutinin absorption and into the subject of the peculiar reciprocal reactions which occur within the paratyphoid group. This would be necessary, if we were to discuss the agglutinin absorption reactions which Winslow and his co-workers record. Our rather extensive observations on these subjects will be published shortly.

Winslow and his co-workers considered the cook who prepared the mayonnaise as the most probable source of the contamination. This would be the natural conclusion, especially if the offending bacterium were considered to be a *B. paratyphosus B.*, in the usual sense.

The rôle of human beings as persistent carriers of the *B. pestis caviae* type is still an open question. The data which we possess indicate that human beings may become carriers. In the case of the more severe types of food infection, at least, some bacteriaemia probably develops and biliary localization is a possibility.

We have identified as *B. pestis caviae* cultures from persons suffering from other conditions than food poisoning. Thus a strain sent to us, labelled as having been isolated by Wherry from the liver of a three-year-old boy with status lymphaticus, is of this type. We have isolated this type from the stool of a woman showing some fever of obscure origin. Her history indicated the existence of a chronic cholecystitis. Two cultures sent us from Panama are also of this type. The clinical history we have thus far obtained suggests that the two cases had a febrile condition of typhoidal character. Both gave "positive" blood cultures. These cases indicate the ability of this bacterium to invade the body and to cause more generalized infections, some of which might terminate in a carrier condition.

Our reason for continuing the use of the term *B. pestis caviae*, as suggested by Theobald Smith, in preference to *B. typhi murium*, is that this description of the guinea pig organism indicated that it was probably a variety distinct from the other paratyphoid types known at that time. Most of the German workers, however, have contended that *B. typhi murium* was essentially a *B. paratyphosus B.* type. Schütze's term "mutton type" is, as he frankly admits, a temporary expedient waiting a better terminology. It does not seem necessary to review the earlier serological work on the strains of rodent origin. The methods, as seen from the standpoint of to-day, were very inadequate and the deductions contradictory. The essential result was not arrived at, namely, that there was a definite and distinct paratyphoid type prevalent among the guinea pig and mouse strains.

In the event of an outbreak, such as we have described, a public administrator is placed between the upper millstone of justice to the storekeeper and the nether millstone of protection of the public. When we were convinced that the bakery shop in question was at fault we faced this dilemma. We did not deem it wise to close the bakery, as such an act is disastrous to the storekeeper, even if it does not bankrupt him. The fact that none but the cream-filled products were involved, and that the bakings of the 6th, 7th and 8th had been eaten without ill effect, indicated that a thorough cleanup and scalding process with a method of protection of the filling during cooling would suffice. Furthermore, nothing had occurred in the preceding years' bakings which were made under the same circumstances. We appreciated, however, that the most real protection was to discover the source of the contamination.

The report of six cases of illness due to the baking of the 9th, a baking which preceded our cleanup, was a source of uneasiness, however. The persistent publicity given the affair by the newspapers, urged on by a publicity agent and the mouth-to-mouth neighborhood gossip, centered attention on the bakery, with the result that the baker, a highly conscientious man, voluntarily discontinued the manufacture of the "cream"-filled products.

After we felt sure of the source of the contamination, we urged him to resume production with precautions to prevent contamination. This he was loath to do, so we arranged with him to prepare the filling on several successive days. We took the finished product to the laboratory, examined it directly and, after incubation, with negative results. He then resumed commercial production and no untoward results have occurred to date, a period of nearly four months.

SUMMARY

The food infection outbreak, here reported, comprised 59 cases. The cause of the outbreak was the contamination of the cream (corn-starch) filling of eclairs and crumb cake. The contamination was found to have been of rodent origin. The causative bacterium was a distinct paratyphoid type, *B. pestis caviae* (*B. typhi murium*—"mutton" type), prevalent in rodents; it has also been isolated from other species. This type, only recently separated with definiteness as a distinct and prevalent paratyphoid variety, is apparently one of the commonest etiological agents of food infection in man.

The *B. pestis caviae* (*B. typhi murium*—"mutton" type), *B. cholerae suis* and *B. enteritidis* are apparently the three varieties most likely to be expected in cases of food infection. One gains the impression that, had the *B. pestis caviae* type been recognized earlier as a distinct paratyphoid type, it might have been found to be the most common of the three varieties.

For bibliography: see the original article.

EFFECT OF STORAGE AND CHANGING SEA WATER ON CONTAMINATED OYSTERS

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Charles Krumwiede, William H. Park, Georgia Cooper, Marie Grund, Charles H. Tyler and Carolyn Rosenstein

In the February issue of the Journal, our experiments on the effect of chlorination on oysters contaminated with typhoid bacilli were reported. Through the exigencies of time and available Journal space the collateral experiments on the effect of changing sea water alone were not presented. These as well as observations on the effect of dry storage were held for report at this time.

When the increase of typhoid fever occurred during the latter part of 1924, it was noticed coincidently in inland as well as seaboard cities. When investigations showed that this increase of typhoid fever was attributable to contaminated oysters it indicated that *B. typhosus* must persist in dry, stored oysters for some time. Experiments were undertaken, therefore, to determine the time of survival of *B. typhosus* under storage conditions.

Oysters for these observations were dredged at West Sayville, Long Island, and sent immediately to the laboratory. The water was obtained from Great South Bay, Long Island.

Two lots of about 80 oysters were contaminated by suspending the oysters in wire crates in tanks containing about 8 gallons of contaminated sea water: Lot A, shown in Table I, in sea water contaminated by the addition of a suspension of feces containing *B. Typhosus;* Lot B, shown in Table 2, in water contaminated with freshly isolated cultures of *B. typhosus*. The tanks were kept overnight at room temperature (18° to 20° C.). The oysters drank actively. They were then removed from the tank for storage. The storage conditions approximated in temperature that of the colder

TABLE 1.

SURVIVAL OF B. TYPHOSUS ON OR IN OYSTERS, CONTAMINATED WITH FECES, DURING STORAGE. FIRST EXAMINATION FEBRUARY 4, 1925

	Estimated number of B. typhosus per		
Days of Storage	cc. of Liquor	cc. of Body Emulsion	Shell
0	254	Not exam.	Not exam.
1	62	110	Not exam.
5	23	10	Not exam.
7	10.4	0.9	3,111
9	20.4	1.85	194
10	2.5	+(<0.08)	142
14	2.5	1.6	Neg.
21	7.6	1.5	Neg.
28	1.3	+(<0.08)	Neg.
35	30.6	0.25	Neg.
41	1.5	+(<0.08)	Neg.
49	0.25	0.25	Neg.

TABLE 2.

SURVIVAL OF B. TYPHOSUS ON OR IN OYSTERS (CONTAMINATED WITH FRESHLY ISOLATED CULTURES OF B. TYPHOSUS) DURING STORAGE. FIRST EXAMINATION FEBRUARY 4, 1925

	Estimated number of B. typhosus per		
Days of Storage	cc. of liquor	cc. of Body Emulsion	Shell
0	37,700	2,118	Not exam.
1	13,870	1,260	Not exam.
5	10,333	1,077	Not exam.
7	7,107	450	Not exam.
9	4,514	473	2,733
14	1,245	515	2,750
21	5	100	Neg.
28	1.6	0.6	100
35	1.4	12	Neg.[1]
41	103	32	Neg.
49[2]	45	26	Neg.

[1] Oysters removed on this date and placed in fresh sea water then showed 160 *B. typhosus* per shell, irregular distribution.

[2] Oysters dead.

months (February and March) of the oyster season. The oysters were kept out-of-doors in a small shed on the north side of a building which was covered with snow. When snow was no longer available they were transferred to an icebox.

The materials used for bacteriological examination were: washings from the shell, using 10 cc. of broth per oyster, the removal of the contaminated material being assisted by rubbing with cotton swab; the liquor; and the body of the oyster emulsified in 5 cc. of broth. The number of *B. typhosus* was estimated from the number of colonies on the plates or from the smallest quantity which, when placed in an enriching brilliant green broth, yielded *B. typhosus* on subsequent plating.

The effect of dry storage on the numbers of *B. typhosus* on or in contaminated oysters is summarized in Tables 1 and 2. The number of typhoid bacilli as shown in Tables 1 and 2 were greatly reduced, but bacilli still could be isolated from both liquor and body after 49 days of storage. Before this the oysters had begun to die and those examined on the last day of the experiment were unfit for food. The number of *B. typhosus* per shell was very low after 2 to 4 weeks' storage; the shells of the more lightly contaminated oysters being negative after 2 weeks, whereas with the more heavily contaminated ones the last positive finding was after 5 weeks' storage.

A parallel series of observations on the persistence of *B. coli* was also carried out using the presumptive broth fermentation tube method. The results of these observations are illustrated in Table 3. In this table as in Tables 1 and 2 all of the examinations are not recorded but only a sufficient number to show clearly the rate of decline in numbers of *B. typhosus* or *B. coli.* In the observations on the survival of *B. coli* only one lactose broth tube was used for each dilution tested. Evidently the actual numbers may have been the estimated numbers given or somewhere between this figure and one-tenth of this figure. Although the contamination with *B. coli* was very heavy and considerable irregularities are noticeable in the results in Table 3, there is a general parallelism in the rate of reduction of *B. typhosus* and *B. coli* on dry storage.

TABLE 3.

SURVIVAL OF B. COLI IN OYSTERS, CONTAMINATED WITH B. COLI, DURING STORAGE. FIRST EXAMINATION FEBRUARY 4, 1925

Days of Storage	Estimated number of B. coli per c.c. Liquor	c.c. Oyster Body Emulsion
0	82,000,000	200,000
1	425,000,000	25,500,000
6	183,000,000	250,000
8	685,000	25,000
10	550	50
21	5,050	505
28	50	5,050
35	50	550
41	1	5.5
49[1]	10	1

[1] Oysters dead.

Another observation on the survival of *B. typhosus* in dry stored oysters was made with some oysters which had been subjected to chlorination. Before chlorination (less than 1.5 p.p.m. of chlorine) the number of *B. typhosus* was 103 per cc. of liquor and 19 per cc. of oyster body emulsion. After storage for 8 weeks (refrigerator) 1 cc. of liquor contained 1.2 typhoid bacilli and 1 cc. of oyster body emulsion gave 0.19 *B. typhosus.*

These experiments show that *B. typhosus* may persist in oysters for a period of time beyond any time of storage which occurs in the trade.

The death of oysters during the period of storage led naturally to the question as to whether a dead oyster was a favorable culture medium for the typhoid bacillus. Killed contaminated oysters were stored at room and icebox temperature and approximately equal portions of body substance were ground in 2 cc. of broth and culture. In oysters contaminated with pure cultures and held at room temperature there was evidence of a slow multiplication approximating 15 times after 7 days, followed by a more rapid rise to approximately 1,000 times after 10 days. At icebox temperature there was a moderate increase to the 7th day, then a slight decrease, the number being approximately the same on the 10th day as on the 1st. In oysters contaminated with stool suspensions, at room temperature the overgrowth of other organisms prevented the isolation of *B. typhosus* by the direct plating method after the 1st day. By enrichment methods *B. typhosus* was recovered up to the 10th day. At icebox temperature *B. typhosus* was isolated up to the 10th day, the last examination made. There was no noticeable increase during this time. These observations indicate the possibility of recontamination by the drip from dead oysters, which contamination would be greater if the temperature were such as to allow growth.

As was to be expected, other workers were impressed by the occurrence of oyster-borne typhoid fever in inland cities and started to investigate the viability of *B. typhosus* on or in oysters.

Kinyoun reports that oysters heavily contaminated with stock cultures of *B. typhosus* yielded *B. typhosus* up to the 15th day, when the observations were terminated.

Jordan likewise contaminated oysters with culture strains of *B. typhosus* and stored the oysters at 5° to 8° C. He isolated *B. typhosus* from the interior of the oyster up to the 24th day. After this the results were negative and the experiment was terminated on the 30th day.

Tonney and White also contaminated oysters with stock cultures of *B. typhosus.* The contamination was extremely heavy. The results with those stored at 45° F. only are quoted. The oysters were opened in the ordinary way, up to the 17th day, so that the typhoid bacilli isolated up to this day may have been from the shell or from the liquor. After the 22nd day the liquor was collected in a way to avoid contamination of the shell. In the case of dead oysters washings were examined. The results were positive to the 60th day, negative thereafter. The oysters remaining after the 39th day were all dead.

Of the earlier observers, Field working in this laboratory showed the persistence of *B. typhosus* for the longest time, *viz.,* 42 days, when the experiment was terminated.

The question of the rate of cleansing which occurs when contaminated oysters drink in successive changes of sea water has been the subject of earlier investigations. We undertook a reinvestigation of this subject because we felt that the means of isolation of *B. typhosus* at our disposal would possibly demonstrate a longer persistence of *B. typhosus* than was indicated by earlier observations. This has been the case, but the conditions of the experiment were not completely satisfactory for the longevity of the oyster and death of the oysters terminated the observations.

The 50 oysters for this experiment were contaminated with typhoid feces and then placed in 4 gallons of fresh sea water. The water was changed 18 times in a period of 24 days. The temperature in the shed where the tank was kept was from 60° to 70° C. and just prior to each change of water samples were taken for examination. Table 4 gives the results of a sufficient number of these observations to indicate the rate of decline in the number of *B. typhosus* present.

TABLE 4.

EFFECT OF CHANGING SEA WATER ON THE NUMBER OF B. TYPHOSUS ON OR IN OYSTERS

Days after contamination	No. of changes of sea water	Estimated number of B. typhosus per cc.		Estimated number of B. typhosus per shell	B. typhosus per cc. of sea water in tank
		Liquor	Oyster body emulsion		
0	0	2,675	213	40,400	12,300
1	1	424	68	9,500	550
3	2	64	42	980	800
4	3	2	0.5	75	Neg.[1]
7	6	8.6	0.5	64	Neg.
11[3]	9	4.	+<0.1	84	Neg.[2]
15[4]	12	7	1.5	74	0.75
21	16	1	2.2	1.2	0.25
24[5]	18	1.6	2.2	3.7	Neg.

[1] Negative in this column means *B. typhosus* not isolated from 20 cc. of water.

[2] Positive on the 8th and 10th days, 7th and 8th change.

[3] Still drinking, grayish slime developing on shells.

[4] Same condition, one dead oyster found.

[5] Remaining oysters dead, slime increased, water had disagreeable odor.

The results indicate that 3 successive drinkings in fresh sea water will result in a reduction of about 99 per cent. of the typhoid bacilli on or in contaminated oysters. This degree of reduction compares favorably with that which was obtained (see previous number of this Journal) with successive treatments with chlorinated sea water and intermediate drinking. There was a difference however; with simple changes of plain sea water *B. typhosus* were isolated from the tank water almost to the end of the experiment. This continued contamination was evidently due to the rinse from contaminated oyster shells and the excretion of contaminating *B. typhosus* from within the oysters. With chlorination, however, we usually failed to isolate *B. typhosus* from the tank water. The exceptions were with heavy contamination in which case the chlorine added was unable to destroy all the typhoid bacilli in the tank water.

The failure to keep oysters alive for a sufficient time in the previous experiment was apparently due to eventual fouling of the water and reduced oxidation of the sea water which was stored in barrels. Permits are granted during the closed season for the removal of oysters from condemned beds to uncontaminated waters for cleansing. It was the intent of the above experiment to give some indication in this regard. Although the indication is that even 3 weeks is insufficient, it seemed desirable to repeat this experiment under conditions more favorable to the oysters' activity. As cold weather had supervened this could not be done, but we plan to carry out such an experiment as soon as the natural waters have warmed sufficiently to insure active drinking.

In the meantime we have investigated the length of time that *B. typhosus* will survive in oysters during the winter months when the oysters are returned to natural waters. The place selected for this experiment was in a bay near a series of condemned oyster beds. The oysters for the experiment were secured from these beds so that the factor of change to different waters might not enter. These oysters were contaminated with typhoid feces. The contamination was comparatively light, 100 *B. typhosus* per shell, 3 per cc. of liquor and less than 0.4 per cc. of body emulsion. The oysters were submerged in the bay water in lots in wire crates and then brought to the laboratory at intervals for examinations.* The results of these examinations are given in Table 5.

TABLE 5

PERSISTENCE OF B. TYPHOSUS IN CONTAMINATED OYSTERS RETURNED TO NATURAL WATERS, 10 OYSTERS EXAMINED AT EACH TEST

No. of days in bay	Date	Temperature of water	Number positive for B. typhosus			Pooled Gills[1]
			Shells	Liquors	Bodies	
0	12-2	....	10	10	9	Pos.
1	12-5	Not taken	0	1	3	Pos.
4	12-9	43° F.	0	1	1	Pos.
10	12-14	36° F.	0	1	1	Pos.
17	12-21	41° F.	0	0	1	Neg.
24[2]	12-28[2]	39° F.	0	0	1	Neg.
31[2]	1-4[2]	39° F.	0	0	2	Neg.
39	1-12	30° F.	0	0	0	Neg
51	1-26	36° F.	0	0	0	Pos.

[1] Gills from the 10 oysters put in brilliant green broth.

[2] Five control oysters (not contaminated but placed in same waters) examined on each date gave negative results.

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*We are indebted to Dr. Rowland G. Freeman for taking care of these details.

The temperature of the water was taken as noted. As a further guide to the probable temperature of the water during the intermediate times we quote the following from the Weather Bureau report:

MEAN DAILY TEMPERATURE (FAHRENHEIT)

December, 1925

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
38°	43°	44°	48°	50°	50°	43°	40°	36°	30°	32°	41°	40°	32°	31°	32°
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
32°	31°	34°	41°	40°	37°	28°	32°	32°	20°	10°	23°	28°	26°	26°	

January, 1926

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
33°	38°	39°	42°	42°	44°	30°	25°	27°	28°	28°	28°	20°	24°	32°	34°
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
34°	46°	44°	42°	46°	30°	22°	26°	23°	28°	32°	22°	14°	30°	39°	

It is probable from the data given that little drinking took place. These observations indicate therefore that should oysters become contaminated during their more inactive period, *B. typhosus* can persist in them for at least 51 days. It was a surprise to us that we should succeed in isolating *B. typhosus* after this length of time considering how light the initial contamination was. The length of time of persistence was equally surprising as there was no reason to believe that *B. typhosus* would survive for this time even in inactive oysters. On the other hand, the conditions might be considered as comparable to dry storage because of the inactivity, and *B. typhosus* will persist for this time in dry stored oysters.

It might be argued that the bacilli isolated came from the relatively unsafe waters in which the oysters were placed. This we believe was not the case as shown by the control (uncontaminated) oysters. The numbers of control oysters were small due to accidental loss of some crates through breaking of the tie-ropes. Even so, we believe that the results indicate that the *B. typhosus* isolated were from the artificial contamination.

Some of these oysters were examined for *B. coli*. A score of *zero* was obtained on oysters yielding *B. typhosus*. It has not infrequently been noted in our experiments that *B. coli* colonies were very few in number or apparently absent on plate media which showed the presence of colonies of *B. typhosus*. Such observations emphasize what one tends to forget—a high *B. coli* score is an indication of danger, a low *B. coli* score is not necessarily an index of safety.

TABLE 6.

POSITIVE FINDINGS FROM ENRICHMENT BROTH (40 SAMPLES) ACCORDING TO DAY OF POSITIVE RESULTS

Days of incubation of brilliant green broth	Specimens grouped according to days of positive findings					
1	12+		28—			
3		10+		18—		
5			10+		8—	
7				6+		2—
10[1]						2—

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[1] Observations continued until the 21st day but no more samples became positive.

In the above work and in that previously reported both the Endo medium and brilliant green agar (Krumwiede) were employed. As was to be expected, the latter frequently yielded positive results, where the former was negative. In addition, a brilliant green broth was used for enrichment. As we knew that the oyster liquor and especially the oyster body emulsion would reduce the activity of the dye, preliminary tests of this factor were carried out. As it would not be feasible to use several dilutions of dye with each of the many samples so as to strike the optimum in each instance, an average optimum was selected. For culturing the oyster body emulsion 1 cc. of a 1 to 1000 solution of brilliant green was added to 50 cc. of broth. For the liquor, 0.25 cc. of the dye solution was employed. The washings from the shell were cultured in 50 cc. of broth to which 0.25 to 0.5 cc. of dye solution (1 to 1000) was added, the amount varying with the quantity of dirt washed from the shells. Veal broth of a reaction of pH.7.2 was employed.

The tables have shown how efficient this procedure has been in isolating the residual few *B. typhosus*. The maximum efficiency of the procedure was not reached, however, till the latter half of our work, that is, until we found how successful repeated platings from the enrichment broth might be. To illustrate this we have tabulated the results of a series of successful enrichments according to the day on which positive results were obtained (Table 6). The 40 positive results are those obtained in 82 attempts at enrichments of *B. typhosus* in the last chlorination experiment we carried out. The table shows how the number of positive results increased on each succeeding examination of the broth.

SUMMARY

B. typhosus persisted in shell oysters stored at refrigerator temperatures for as long as the oysters lived, a period of 41 days. The oysters dying after this time were examined on the 49th day of storage and also yielded *B. typhosus*. The *B. typhosus* on the shells of stored oysters were apparently dead after 14 to 40 days, the length of persistence depending on the degree of contamination. A dead oyster serves as a culture medium and the number of *B. typhosus* will increase if the temperature is satisfactory for multiplication. Repeated changes of fresh sea water resulted in a rapid diminution of the number of *B. typhosus* on or in oysters, the diminution reaching about 99 per cent. after 3 changes of water. *B. typhosus* was isolated up to 21 days, during which time the water was changed 16 times, after which the remaining oysters died. The degree of purification following 3 changes of fresh sea water approximated that observed with a similar number of treatments of chlorinated sea water. There is a difference, however; in most instances the *B. typhosus* in the chlorinated water was apparently killed. Contaminated oysters were placed in natural waters the temperature of which was low enough to largely inhibit drinking. The oysters contained *B. typhosus* after 51 days in these waters, the last observation made. In some of these experiments as well as in some of those described in our previous report, note should be made of the prolonged persistence of minimal numbers of *B. typhosus* under different conditions. This prolonged persistence emphasizes the fact that apparently the only safe oyster is one which has been protected from any contamination with fecal pathogens for at least some months prior to harvesting. The actual time can only be determined by further careful experiments.

For Bibliography: See the original article.

THE CHLORINE TREATMENT OF CONTAMINATED OYSTERS

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The great success which has attended the use of chlorine for the purification of water supplies has led to an attempt to apply a similar procedure to the purification of contaminated oysters. Wells, on the basis of experimental observations, has advocated the use of chlorinated sea water treatment of oysters and believes that contaminated oysters thus treated may be rendered safe for consumption.

The following quotation from Wells (1920) indicates the mechanism on which he bases his procedures:

Briefly stated, this method of purification consists of nothing more than assuring conditions of cleanliness under which the oyster can, by its natural function, remove any pollution received from the water. Ordinarily the oyster is very active in filtering out and digesting fine particles which are drifting in the water. If kept in clean surroundings, the oyster is just as active in returning these substances into the water. Under ordinary conditions an oyster passes 50 gallons of water a day through his gills, and a particle of food deposited on those gills will pass on to the mouth and be eliminated from the oyster within 5 hours. If conditions are maintained such that pollutions are removed, and new ones are not permitted to enter the oyster, it is possible to cleanse a polluted oyster within a remarkably short period. Twenty-four hours has been found sufficient under ordinary conditions of practice. With slightly polluted oysters less time is required than for grossly polluted oysters. The latter, which it would not be proposed to treat, might require a longer period than 24 hours.

From this standpoint the apparent value of chlorination rests on its efficiency in sterilizing the available water, the exterior of the oyster shells and the material excreted from the oyster. Indirectly it depends on the completeness of the excretion of contaminating organisms by the oyster itself. For a consideration of these aspects the experiments given below were carried out.

The following is an outline of the source of materials and the methods employed in our experimental tests of the efficiency of the chlorine treatment of oysters.

The oysters were specially dredged for us through the courtesy of one of the local oyster concerns.* They came from Great South Bay, Long Island. This insured fresh active oysters and the relative purity of the waters from which they were dredged helped to reduce the frequency and numbers of bacteria which would interfere with the isolation of specific types. The oysters were "reasonably clean." The water also was obtained from Great South Bay.

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* We are indebted to Mr. Hugh Taylor, Director of the Bureau of Foods and Drugs, and to Mr. Richard Ross of the same Bureau for arranging these details.

The oysters were contaminated on arrival at the laboratory by immersing them and allowing them to drink in sea water contaminated with feces containing *B. typhosus.* This procedure seemed more desirable than the use of culture strains. It approximates the natural conditions of contamination and leaves no question as to acquired or lessened resistance through cultivation. The oysters always drank freely and sedimentation resulted in the contamination of the shells.

For chlorination a concentrated solution of chlorine gas in tap water was employed. This was added in appropriate quantities to sea water to give the desired chlorine content. The oyster sea-water ratio was adjusted to within the recommendations quoted above. The details as to amount of chlorine, time of treatment, etc., are given with each of the experiments. The content of chlorine was determined by titration.

The oysters after contamination or treatment were prepared for bacteriological examination with the following possibilities in mind: Were *B. typhosus* present on the shell, in the liquor or, as far as could be determined, were they in the body of the oyster. Three sets of materials were therefore examined, washings from the shell, the oyster liquor and an emulsion of the body in broth. The first was obtained by scrubbing the shell with a cotton swab, in the later experiments with a tooth-brush using a constant quantity (15 cc.) of broth for each oyster. This served as an estimate of the degree of shell contamination. The oyster was then dipped momentarily in boiling water and drained, then opened and the liquor collected. The gills were then cut from the body and the body ground in a mortar with 5 cc. of broth. The volume of the oyster body emulsion averaged about 10 cc. Naturally this emulsion would contain traces of liquor. Comparative observations with washed and unwashed bodies and bodies treated with strong chlorine solutions indicate that the bacilli obtained from the body emulsion were mostly either in the mucus covering the body or in its interior. Actually this point is only of relatively academic interest, the practical point being whether *B. typhosus* were present or not.

The bacteriological examinations were planned to take advantage of the newer refined methods of isolating *B. typhosus,* thus making it possible to discover *B. typhosus* in many instances where the findings would have been negative with the older methods. The washings from the shell, the liquor and the ground oyster body suspension were separately inoculated in measured amounts and spread on brilliant green agar plates. From 0.1 to 0.4 cc. of the undiluted samples generally were used per plate, but where the necessity was indicated, dilutions were employed and 0.01 or 0.001 per plate inoculated; 6 to 10 plates were employed for each sample. After incubation the number of *B. typhosus* colonies per plate were counted. The identification was based on the results of inspection supported by slide agglutination and in doubtful cases confirmed by fishing to triple sugar medium and making further agglutination tests. Complete serological tests were made wherever the results were still indefinite. In addition to the direct plating of the material, the remainder of the sample was inoculated for enrichment into brilliant green broth. The broth was plated on brilliant green media at 24 hours and on later days, in some instances up to 3 weeks. The first positive findings of the broth were found on the 1st up to the 10th day inclusive. The necessity of repeated examinations is very apparent.

The figures given in the tables are based on the colonies per plate with correction for the volume employed or on an amount which yielded *B. typhosus* when added to brilliant green broth.

The number of oysters examined during or at the end of the chlorination experiments has varied. In the earlier tests only 2 oysters were examined at each test period. This resulted in some apparent discrepancies due to the unevenness of the distribution of *B. typhosus*, and the number examined was increased to 4. In the last experiment 9 oysters were examined at each time.

Chlorinated water was tried at different temperatures in some of the experiments to determine whether this would influence the effect of the chlorine and to determine the differences in the rate of chlorine fall.

The experiments are given below in the order in which they were carried out, in most instances with an accompanying table giving the details of the test.

Experiment 1 was a relative failure in that an excessive overgrowth of *B. aerogenes* types prevented the isolation of *B. typhosus*, in all the earlier tests in the series. The experiment is significant, however, in that with a treatment in chlorinated water (total available 36.3 parts per million at the start) we were able to isolate *B. typhosus* from the shell washings at the end of 27 hours in the bath. During this time the total chlorine had fallen from 36.3 to 1.2 parts per million. The temperature of the bath was from 9° to 14° C. during this period.

In chlorination experiment 2 (Table I) the contamination of the shell was heavy, 12,300 *B. typhosus* per shell, but the chlorine content was high, 32 parts per million. The test at the end of an hour showed a sharp fall in the number of typhoid bacilli, partly due to the wash effect of the chlorinated water removing the sediment from the shells. At the end of 5 hours there was no marked further decline in numbers, indicating that many of the bacilli were protected from the action of the chlorine. At the end of 24 hours in the cold tank the chlorine had dropped from 32 parts per million to 1.5 parts and in the warmer tank from 32 parts to 0.5 parts and typhoid bacilli were isolated in considerable numbers from all the shells tested. It

TABLE 1.

EFFECT OF CHLORINE ON THE NUMBERS OF B. TYPHOSUS ON THE SHELLS OF CONTAMINATED OYSTERS

Tank	Time of Treatment	Temp.	Chlorine p.p.m.	B. typhosus per shell
A	0	20° C.	32.1	12,300
	2 hrs.	13° C.	12.6	450
	3 hrs.	12° C.	8.9	570
	4 hrs.	12° C.	6.8	150
	24 hrs.[3]	6° C.	1.6[1]	60
B	0	20° C.	32.1	12,300
	2 hrs.	20° C.	11.8	2,800
	3 hrs.	20° C.	7.9	130
	4 hrs.	21° C.	5.3	160
	24 hrs.[3]	25° C.	0.5[2]	70

[1] In a control tank without oysters the chlorine was 21.6 p.p.m.

[2] In a control tank without oysters the chlorine was 10.2 p.p.m.

[3] Drinking probably took place some time before the 24th hour.

NOTE—Several examinations of the chlorinated sea water were made after the contaminated oysters were placed in the tank. These gave negative results.

should be noted, however, that the chlorine content fell between the 5th and 24th hour to a point where drinking and recontamination of the shells by excreted *B. typhosus* could have occurred. Deductions as to effect of the chlorine on the initial shell contamination must take this into consideration.

In view of the unfavorable results of the second experiment the experiment 3 (Table 2) was carried out with a relatively light contamination. The concentration of chlorine was approximately the same as the previous experiment. Typhoid bacilli were recovered from the shells of the oysters in both tanks at the end of 5 hours. At the end of 26 hours in the case of the tank kept at lower temperature no *B. typhosus* was recovered. It should be noted how the chlorine persisted in the latter tank. In the warmer tank the oysters were drinking at the 23rd hour and *B. typhosus* was recovered from the shell. This experiment shows no apparent immediate decrease in the number of *B. typhosus*. This apparent discrepancy is probably due to irregularity in contamination and to the accidental selection of oysters of low contamination at the beginning of the experiment.

TABLE 2.

EFFECT OF CHLORINE ON THE NUMBERS OF B. TYPHOSUS ON THE SHELLS OF CONTAMINATED OYSTERS

Tank	Time of Treatment	Temp.	Chlorine p.p.m.	B. typhosus per shell
A	0	19° C.	34.9	18
	1 hr.	14° C.	22.1	80
	3 hrs.	7.5° C.	15.6	70
	5 hrs.	5° C.	14.4	85
	24 hrs.	3° C.	8.1	10
	28 hrs.	5° C.	3.5	neg.
	48 hrs.	6° C.	2.1[1]	neg.
B	0	19° C.	34.9	18
	1 hr.	20° C.	16.5	100
	3 hrs.	20° C.	7.9	310
	5 hrs.[3]	21° C.	4.6	55
	24 hrs.	18° C.	1.1[2]	40
	26 hrs.	......	....	neg.

[1] A control tank without oysters contained 20 p.p.m. of chlorine.

[2] A control tank without oysters contained 7.9 p.p.m. of chlorine.

[3] Drinking began some time after the 5th hour.

NOTE.—B. typhosus could not be isolated from the chlorinated water after introduction of the contaminated oysters.

In experiment 4 (Table 3) a heavier contamination occurred than was expected. The concentration of chlorine employed was 15 parts per million. The results were similar to those obtained in the previous experiments, a marked initial decrease in the numbers of *B. typhosus* at the 1-hour test and very little apparent reduction in the tests subsequent to that. It should be noted that the contamination was so heavy that even the supernatant sea water was not rendered free of *B. typhosus*.

TABLE 3.

EFFECT OF CHLORINE ON THE NUMBERS OF B. TYPHOSUS ON THE SHELLS OF HEAVILY CONTAMINATED OYSTERS

Tank	Time of Treatment	Temp.	Chlorine p.p.m.	B. typhosus per shell
A	0	16° C.	15.8	143,500
	1 hr.	12° C.	6.9	5,390[2]
	3 hrs.	7° C.	2.4	1,450
	5½ hrs.[1]	5° C.	1.4	3,020
	24 hrs.	......	....	820
	27 hrs.[1]	4° C.	0.4	2,890
B	0	16° C.	15.8	143,500
	1 hr.	16° C.	6.0	4,150[2]
	3 hrs.	16° C.	0.7	1,250
	5½ hrs.	17° C.	0.3	1,440

[1] B. typhosus isolated from the tank water.

[2] Reduction partly due to rinse effect, see later experiments.

In experiment 5 (Table 4) the concentration of chlorine approximates that of the previous experiment (14 parts per million), but the contamination was very much lighter. *B. typhosus* was present on the shells at the end of an hour, and on the shells, in the liquor and bodies at the end of 6 hours. The oysters drank after the 1st hour.

TABLE 4.

EFFECT OF CHLORINE ON THE NUMBERS OF B. TYPHOSUS ON THE SHELLS OF CONTAMINATED OYSTERS

Time of Treatment	Temp.	Chlorine p.p.m.	B. typhosus per Shell
0	16° C.	14.5	395
1 hr.[1]	14° C.	3.9	94
3 hrs.	12° C.	0.9	65
5 hrs.	10° C.	0.3	54

[1] Oysters drinking between 1st and 3rd hour.

These experiments were disappointing and in themselves raised a grave question as to the efficacy of any chlorine treatment, as *B. typhosus* was isolated from the shells with only one exception before the oysters started to drink even though the initial chlorine content was as high as 30 parts per million in 2 of the experiments, that is, the degree of exposure was many times greater than that recommended by Wells.

Experiment 6 (not given in tabular form) really belongs to the above series. Although originally planned to determine the effect of successive chlorinations with intermediate drinking periods it was not continued. The oysters showed a minimum contamination, so slight, that even the rinse effect of sea water apparently was sufficient to clear the shells. In spite of this, *B. typhosus* was obtained from the bodies after at least 3 to 21 hours' drinking.

Experiment 7 (Table 5) was carried out with a slightly heavier contamination. Three exposures to chlorine were made, showing 8 parts per million, 24 hours later 12 parts, and again after 24 hours 12 parts per million. This experiment from the standpoint of the period of drinking as advised by Wells can only be considered as a double chlorination. However, typhoid bacilli could be isolated from the shells at every test up to 4 hours after the last chlorination. The liquors gave negative results 24 hours after the second chlorination but the bodies were still positive. This indicates that the flushing effect of the drinking may not result in the elimination of even small numbers of typhoid bacilli.

TABLE 5.

EFFECT OF SUCCESSIVE CHLORINATIONS ON THE NUMBERS OF B. TYPHOSUS ON AND IN OYSTERS

	Treatments		Chlorine	B. typhosus, Number per		
No.	Time of	Temp.	p.p.m.	Shell	cc. of Liquor	cc. of Body Emulsion
1..........	0	16° C.	8.7	130	1.6	0.5
	1 hr.[1]	14° C.	1.2	35[3]	..	...
	3 hrs.	13° C.	0.5	11	...	...
	6 hrs.	11° C.	0.2	20	0.12	0.25
	24 hrs.	8° C.	0	4.3	neg.	+< 0.06
2..........	0	19° C.	12.7	...	...	...
	6 hrs.[2]	20° C.	0.4	6.9	0.19	0.19
	24 hrs.	18° C	0	2.5	neg.	0.125
3..........	0	19° C.	12.0	...	...	...
	4 hrs.	19° C.	1.4	2.5	neg.	+< 0.06

[1] Drinking between 1st and 3rd hour.
[2] Drinking not observed, opportunity during 18 hours.
[3] Dipping in plain sea water reduced the count to 19 B. typhosus per shell.

Owing to this failure of two exposures to moderate amounts of chlorine, experiment 8 (Table 6) was carried out with larger amounts. From 22 to 25 parts per million were used and a total of 4 exposures was given. The oysters were lightly contaminated. Typhoid bacilli were found on the shells up to 4 hours after the last (4th) chlorination but not 24 hours after

TABLE 6

EFFECT OF SUCCESSIVE CHLORINATIONS ON THE NUMBERS OF B. TYPHOSUS ON AND IN OYSTERS

	Treatments		Chlorine	B. typhosus, Number per		
No.	Time	Temp.	p.p.m.	Shell	cc. of Liquor	cc. of Body Emulsion
1..........	0	10° C.	22.6	197	37	2.8
	4 hrs.[1]	11° C.	4.6	0.6[4]	0.24	0.25
	24 hrs.	11° C.	0.2	4.4	0.31	+<0.06
2..........	0 hrs.	......	22.4	...	...	...
	4 hrs.[1]	11° C.	7.6	1.25	+<0.06	.06
	24 hrs.	12° C.	0	1.88	0.125	0.37
3..........	0	......	25.2	...	...	...
	4 hrs.	12° C.	8.0	3.75	1.13	0.44
	24 hrs.[2]	12° C.	1.4	1.88	0.3	+<0.06
4..........	0	......	22.9	...	...	...
	4 hrs.	12° C.	15.9	0.6	+<0.06	+<0.06
	24 hrs.[3]	11° C.	0	neg.	0.06	+<0.06

[1] Oysters drinking some time after 4th hour.
[2] Only a very short drinking period before rechlorination
[3] Probably had drunk for only short period.
[4] Dipping in plain sea water reduced the count to 30 B. typhosus per shell.

the 4th chlorination. The rate of fall of the chlorine during the first two treatments indicates that drinking was possible for at least a considerable part of the night. The only actual observation was that they were drinking in the morning. After the 3rd treatment the drinking opportunity was very short and the retention of chlorine after the 4th prevented drinking. This lessening of the rate of loss of chlorine after each treatment is noteworthy in other experiments in this series and is undoubtedly due to the gradual lessening of the organic matter through the rinse effect of the changes of sea water.

Apart from the shortness of the drinking period after the 3rd chlorination and its absence after the 4th, the experiment emphasizes again the weakness of chlorination as a means of freeing the exterior of the shell of the oyster of *B. typhosus,* in spite of amounts of chlorine and periods of action many times greater than recommended by Wells. With the slightly heavier pollution both the liquor and the body emulsion yielded *B. typhosus* to the end of the experiment. As in the previous experiment a few bacilli remained in spite of the drinking.

Experiment 9 (Table 7) was planned as a final test of all the factors. It was especially desired that by actual observation there would occur a drinking period after each chlorination in excess of 12 hours. It will be noted that the exigencies of the experiment resulted in much longer times in some instances. The almost constant temperatures were maintained by a continually flowing jacket of tap-water from the street main. This left no question as to dropping temperature on cold nights with inhibition of

TABLE 7

EFFECT OF SIX SUCCESSIVE CHLORINATIONS ON THE NUMBERS OF B. TYPHOSUS ON AND IN OYSTERS

Treatments No.	Time	Chlorine p.p.m.	Drinking Observed	B. typhosus numbers of per (with number of positive oysters in parentheses) Shells	cc. of Liquor	cc. of Body Emulsion
1..........	0	10.9	...	248(9)	1.5(8)	+<0.04(4)
	4¼ hrs.	3.	No	...	...	...
	6 hrs.	1.4	Yes	...	...	...
	23 hrs.	0.2	Yes	2(9)	0.3(7)	+<0.04(6)
2..........	0	10.8	...	...	...	...
	3¾ hrs.	3.5	No	...	...	...
	5¼ hrs.	2.3	Yes	...	...	...
	6¼ hrs.	1.9	Yes	...	...	...
	23 hrs.	0.7	Yes	+<0.08(8)	0.3(8)	+<0.04(8)
3..........	0	10.9	...	...	...	...
	3¾ hrs.	5.5	No	...	...	...
	4 hrs.	...	Yes	...	...	...
	5¼ hrs.	...	Yes	...	...	...
	6¼ hrs.	4.1	Yes	...	...	...
	23 hrs.	2.8	Yes	...	...	...
	46 hrs.	1.8	Yes	1.3(9)	0.09(8)	+<0.04(4)
4..........	0	10.8	...	...	...	...
	6½ hrs.	3.9	No	...	...	...
	24 hrs.	0.7	Yes	...	...	...
	30 hrs.	0.5	Yes	...	...	...
	48 hrs.	Trace	Yes	+<0.08(6)	+<0.04(3)	+<0.04(1)
5..........	0	10.7	...	...	...	...
	5½ hrs.	2.7	No	...	...	...
	23 hrs.	0.3	Yes	...	...	...
	28 hrs.	0.2	Yes	...	...	...
	72 hrs.	0	Yes	4.7(7)	0.6(6)	+<0.04(6)
6..........	0	9.7	...	...	...	...
	4 hrs.	1.6	No	...	...	...
	6½ hrs.	0.9	Yes	...	...	...
	24 hrs.	0.3	Yes	1.3(5)	0.2(5)	+<0.04(8)

NOTE.—Dipping in plain sea water reduced the count to 57 B. typhosus per shell.

drinking. The experiment serves as a sort of *reductio ad absurdum* in that *B. typhosus* was isolated from the shells, from the liquor and from the bodies after a number of chlorinations and a period of treatment in excess of anything expedient in the trade.

A study of this table with special reference to the number and lengths of the drinking periods gives a discouraging view of the efficiency of the cleansing action of drinking, assuming that recontamination of the interior from the shells was not the factor. Whatever interpretation one may make, the practical fact remains that in this as in the previous experiments, a few typhoid bacilli persist inside of the oyster as well as upon the shells.

Conclusions

The chlorination treatment of contaminated oysters will result in a marked diminution in the number of *B. typhosus,* but even 6 successive treatments may not rid the oysters of the contaminating pathogens. The process cannot be recommended therefore in any sense as a reliable means of "sterilizing" contaminated oysters and thus rendering them safe for consumption. The results indicate that safe oysters can only be produced by growing them in waters uncontaminated by human feces.

A GRAM-POSITIVE ANAEROBIC BACILLUS—PRESUMABLY THE CAUSE OF A FATAL FOOD INFECTION.

Lucy Mishulow and M. Edw. Marten.*

A gram-positive anaerobic bacillus was isolated from the liver and spleen of a patient who died within 24 hours after eating in a restaurant. The patient complained of thirst soon after eating, then vomited. Shortly after that he developed psychosis and was sent to a hospital where he died within 24 hours after admission.

The autopsy showed as follows:

Some petechial hemorrhages of the skin of the body particularly on the left side of the abdomen.

The lungs showed intense congestion and edema with very marked petechial hemorrhages along the pleura.

There were intense hemorrhages retroperitoneally throughout the abdominal cavity particularly in the mesentery.

The bowels showed considerable hemorrhages.

The organism isolated from this patient is gram-positive in young cultures; but after 2 to 3 days incubation many gram-negative forms are found. It is a spore-bearing, capsulated and moderately motile bacillus, which grows only under anaerobic conditions. The bacilli in young cultures are about the size of the vibrion septique are fairly regular but in tissues and also in old cultures there are long filaments present.

It grows rapidly in nutrient media but only in the presence of a fermentable sugar.

Semisolid glucose-agar shake-cultures are almost entirely disrupted by the large amount of gas formed in 24 hours at 37°C. leaving a flocculent sediment in the bottom of the tube.

Glucose-broth is heavily clouded and very frothy after 24 hours incubation at 37° C. But on further incubation, the broth clears leaving a very heavy flocculent precipitate.

Spores are readily formed in the above medium; after 24 hours incubation the sediment contains numerous spores. They are oval and mostly central, although there are some subterminal spores. Some of them are larger in diameter than the bacilli thus giving them a spindle, or chlostridium appearance.

The colonies on glucose-agar are small, gray, and at first appear like a fine precipitate. On longer incubation they increase in size, but do not become very large even after two weeks incubation.

On blood-agar the growth is very delicate. The colonies are small, flat, transparent and non-hemolytic.

Litmus milk is acidified and coagulated after 2 to 3 days incubation at 37°C. with the production of gas which gives the clot a worm-eaten appearance.

Brain-medium is not blackened, and cooked-meat medium is not digested.

It does not grow in peptone-water even in the presence of a fermentable sugar.

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It ferments a large number of sugars. Table I gives the results of the fermentation reactions in extract agar, Ph 7.8 to which 1% of the test sugar was added. The inoculations were made from the sediment of a 24 hour glucose broth culture, a loopful of which was stabbed into the depth of the tube. In all the sugars that were fermented the reaction was complete after 24 hours incubation at 37°C. except in xylose, inulin, and glycerin where the reaction was not complete until 48 hours incubation. Where the reaction was negative the tubes were incubated for 10 days to rule out slow fermentation.

TABLE 1

FERMENTATION REACTIONS IN EXTRACT-AGAR—1% OF TEST SUGARS —ANDRADE INDICATOR

	Glucose	Levulose	Galactose	Lactose	Saccharose	Maltose	Raffinose	Dextrin	Salicin	Arabinose	Xylose	Inulin	Glycerin	Mannitol	Dulcital	Sorbitol
Acid...	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
Gas....	++	++	++	++	++	+	++	++	++	++	+	+	+	–	–	–

PATHOGENICITY

This organism is not pathogenic for guinea pigs or rabbits. But a rabbit that was inoculated intravenously with a glucose-broth culture, killed 15 minutes after the injection and incubated at 37°C. overnight, showed characteristic post-mortem changes. There was bleeding from the nose and both ears were bright pink due to dilation of the superficial blood vessels. The subcutaneous tissues were intensely injected over the chest and thighs also the intestines showed areas of injection.

The bacilli were isolated from the blood, lungs, liver and spleen of the rabbit.

The organism is moderately pathogenic for mice. Table II gives the results on a series of mice that were injected intraperitoneally and subcutaneously and also were fed on the culture.

The results show that the culture is more pathogenic when inoculated subcutaneously than introperitoneally also that ½ to 1 c.c. of the culture when fed to mice will kill them in 2-4 days producing lesions that are similar somewhat to those found in the patient at autopsy.

There was little difference in the virulence between a 1 day and 14 day old culture thus indicating that there is apparently no extracellular toxin produced in culture.

This organism differs from any of the known anaerobes that are pathogenic when ingested, both in its biochemical and cultural characteristics, and also in the lesions that it produces in mice. Stoddard[1] described an organism very similar in morphology and biochemical characteristics which he isolated from a gas gangrene wound. He also found it non-pathogenic for guinea pigs and rabbits but he did not test it on mice and appeared to be doubtful as to its pathogenicity.

Unfortunately the food that the patient had eaten was not available for examination and therefore it could not be definitely proven that it was the cause of the infection. But judging from the results of feeding mice on the culture it seems possible that the ingestion of a large number of the bacilli in food might be the cause of a fatal infection.

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[1] Stoddard, J. L.—Bacillus multifermentans tenalbus. Lancet I:12. Jan. 4, 1919.

TABLE 2.

PATHOGENICITY FOR MICE

Mouse	Culture	Age of Culture	Method of Inoculation	Amount Inoc.	Result	Pathological Findings	Remarks
1.	Cooked Meat	24 hrs.	Introp.	½ c.c	Died in 4 days	Intestinal Injection	
2.	“ “	7 days	“	1 c.c.	Died in 8 days		
3.	“ “	7 days	“	½ c.c.	7 days	Hemorrhage from tail	
4.	Glucose broth	24 hrs.	“	1 c.c.	5 days	“ “ “ Subcutaneous & Peritoneal injection	
5.	“ “	24 hrs.	“	½ c.c.	Sick recovered		
6.	“ “	24 hrs.	Subcut.	0.2 c.c.	Died in 6 days		
7.	“ “	48 hrs.	“	1 c.c.	Died in less than 24 hrs.	Subcutaneous and intestinal inj.	
8.	“ “	14 days	“	1 c.c.	Died in less than 24 hrs.		
9.	“ “	14 days	“	½ c.c.	Died in less than 48 hrs.		
10.	“ “	14 days	“	0.2 c.c.	Died in less than 72 hrs.	Hemorrhage from tail	
11.	“ “	24 hrs.	Fed (Pipette)	0.2 c.c.	Remained well		
12.	“ “	24 hrs.	Fed on Bread	1 c.c.	3 to 4 days	Paws, legs and tail bright red, also subcut. inj. over the chest	Bacillus isolated from liver and spleen
13.	“ “	48 hrs.	“	1 c.c.	3 to 4 days	Paws bright red	Bacillus isolated from liver and spleen
14.	“ “	48 hrs.	“	½ c.c.	48 hrs.		Bacillus isolated from liver spleen and intestine

THE DANGER OF DECOLORIZING VACCINE VIRUS

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Charles R. Tyler and Charles Krumwiede

In 1918 one of us in collaboration with Fielder and Watson reported observations on the use of brilliant green in vaccine virus. It was shown that the bactericidal action of this dye in concentration of 1–10,000 was a very potent means of obtaining a relatively rapid reduction of the bacterial content of vaccine virus or even obtaining practical bacterial sterility. This dye in this concentration was found to have no measurable effect on the virus itself.

These facts had been applied to some extent in the manufacture of our vaccine virus but the use of the dye had not been made a routine procedure because of the hesitancy of physicians to use a virus having a green color.

Due to the recent great demands for vaccine virus the expediency of the use of the dye led to its greater use and today we are employing it as a routine.

Coplans in 1922 published observations verifying our findings. He also noted that the green color was a drawback from the practical standpoint and investigated means of ridding the finished vaccine of the color. He recommended the use of sodium hydrosulphite as a decolorizing agent and stated that virus so treated remained potent during a period of fifteen months' storage at a temperature of 45° to 55° F. He stated, however, that some care is needed in the employment of this decolorizing agent, as an excessive amount of sodium hydrosulphite is capable of acting as a vaccinicide.

In extending our use of brilliant green we naturally had complaints about the color, and the recommendation of Coplans seemed worthy of observation and possibly of adoption.

Three lots of vaccine were decolorized by adding sodium hydrosulphite in a concentration of 0.25 gram per 100 c.c.; a quantity considered safe by Coplans. Clinical tests on children as well as rabbits were made over a period of three months; the vaccine being stored at 4° F. The continuously satisfactory "takes" seemed to warrant our marketing these viruses. They were accordingly filled in capillary tubes and vials and distributed.

Shortly we were in receipt of many complaints of vaccination failures. Because of the unfortunate failure of many physicians to keep a record of the lot or preparation number of the vaccine virus they use, there was a considerable delay before we became cognizant of what was wrong. It developed, however, that the decolorized viruses were at fault. This seemed inexplicable in the light of the fact that we were still obtaining 100 per cent "takes" in children and good takes on rabbits with the viruses diluted to 1–1,000.

There was this difference, however: the virus we were employing come directly from cold storage whereas the marketed virus had a delivery time of 24 to 48 hours during the warm weather of June. It seemed therefore that the virus must be defective in its resistance to higher temperatures. The available decolorized lots 361 and 363 were placed in the incubator at 37° C. for 24 hours and longer and then retested. As a control two other lots of about the same age and strength were simultaneously incubated. Reference to the table will show that the viruses containing the hydrosulphite became

RESULTS OF RABBIT VACCINATIONS WITH VACCINE VIRUS BEFORE AND AFTER INCUBATION AT 37° C.

Number and Character of Virus	Relative Potency—Rabbit Test Not incubated	Incubated 24 hours	48 hours	72 hours
361—Brilliant green and hydrosulphite	1-1000+++	1-10—	1-10—	1-10—
363—Brilliant green and hydrosulphite	1-1000+++	1-10—	1-10—	1-10—
366—Brilliant green	1-1000+++	1-1000+++	1-100+	1-10—
375—Brilliant green	1-1000+++	1-1000+++	1-10+	1-10—
388—Glycerol and Carbolic only	1-1000++++	1-1000++±	1-1000++±	1-1000++*
388—Brilliant green	1-1000++++	1-1000++±	1-1000++±	1-1000++±
393—Glycerol and Carbolic	1-1000++++	1-1000++++	1-1000++++	1-1000++++
393—Hydrosulphite 0.25 gm. per 100 cc.	1-1000++++	1-1000++++	1-10+±	1-10+
393—Hydrosulphite 0.025 gm. per 100 cc.	1-1000++++	1-1000++++	1-100++	1-10++
393—Brilliant green	1-1000++++	1-1000++++	1-1000+++	1-1000++
393—Brilliant green and hydrosulphite	1-1000++++	1-1000++	1-10—	1-10—

* After 96 hours the glycerol carbolic virus failed to take at a 1-100 dilution whereas the brilliant green virus still gave a moderate take at 1-1000.

Tests were made by cutaneous insertion of virus in dilutions of 1-10, 1-100 and 1-1000. Where takes occur they are recorded in the table in terms of the highest dilution giving a take and the degree of reaction with this dilution.

practically impotent within 24 hours, whereas the viruses containing brilliant green deteriorated to the same extent in 72 hours.

The pulp from two calves was collected and ground in glycerol-carbolic. These viruses were utilized for comparative experiments. The results are given in the table. In comparing the results it must be kept in mind that some irregularities will necessarily develop due to the varying susceptibility of different rabbits. This probably explains the apparent contradiction that with virus 388 the brilliant green virus did not deteriorate as rapidly as the glycerol-carbolic virus, whereas with virus 393 the opposite result was obtained. Continued observations have shown definitely that brilliant green in the concentration employed has no appreciable vaccinicidal action.

The experiments with virus 393 show very difinitely the vaccinicidal activity of sodium hydrosulphite. This virus had a much greater initial potency than viruses 361 and 363. For this reason naturally complete loss of potency did not occur so rapidly.

The last observation would seem to indicate that the combination of brilliant green and sodium hydrosulphite was more markedly vaccinicidal than was the hydrosulphite alone. As noted above, rabbits vary in their susceptibility and conclusion cannot be drawn from the one observation given.

SUMMARY

The addition of sodium hydrosulphite to brilliant green treated vaccine virus as a decolorizing agent is a dangerous procedure. If the virus so treated is kept at low temperature no deterioration may be noted. At higher temperatures, especially as they approach 37° C., the sodium hydrosulphite (or its cleavage products) shows a distinct vaccinicidal activity and tends to quickly destroy the potency of the virus. Its addition to vaccine virus which is to be marketed is therefore to be condemned.

For bibliography see the original article.

VACCINIA

Caroline R. Gurley

Fifteen filtration experiments with vaccine virus are reported upon, together with control and sub-experiments.

Technic

The samples of vaccine used were from the calf; they represented preparations used for human inoculation, after they had passed the prescribed bacteriological tests and had been recently tested for full activity. Each preparation consisted in a mixture of 0.4 gram of pulp with 4.6 grams of the following diluent: Glycerin 50 cc., water 49 cc., phenol 1 cc. This mixture was pressed through a brass sieve (40-to-the-inch mesh) and then through a sieve of a hundred to the inch mesh. For filtration the vaccines were diluted in most cases with physiological saline solution, usually after reducing the finely divided skin to a state of still finer division by grinding with sand. The degree of dilution employed is stated in terms of dilution of the pulp,—not in those of dilution of the vaccine as given above. The dilutions ranged from 1:12 to 1:50 or 1:60, the usual dilution being about 1:25. When the filtrate was to be concentrated by evaporation in vacuo, 0.5 saline solution (instead of 0.85) was employed.

Berkefeld filters (Grade V) were employed. Every suspension of virus was inoculated, before filtering, with an emulsion of B. prodigiosus (occasionally with staphylococcus instead) to test the integrity of the filter; cultures of the filtrate (1 cc. on each slant) were made to determine presence or absence of the organism.

Clogging of Berkefeld.—Great difficulty arises from clogging of the filter by the finely divided skin present in the virus mixture, though this trouble is much less when the virus is not sandground. The following ways of clearing the material were tried (a) long centrifuging; (b) filtration through paper, paper pulp, shredded asbestos, alundum cups, and sand,—the last used by itself or mixed with a small amount of animal charcoal. The first four methods of filtering failed because of quick clogging and sand did not remove the fine particles. Brushing the surface of the candle with a stiff brush is not applicable during positive pressure work, though a help during suction work. During long centrifuging (1½ hours at 2040 revolutions) the organisms appeared to be carried down in large proportion by the sedimenting skin-particles. The addition to the cloudy fluid, of barium sulphate and also of starch was tried with two portions of virus, in comparison with a third portion which received nothing. The tube containing the sulphate cleared more quickly and thoroughly on centrifuging, than the other two, between which there was little difference. The supernatant fluid containing the virus from all three was passed through a Berkefeld V with a positive pressure of somewhat less than 50 pounds, (the desired pressure aimed at), and then inoculated intradermally into rabbits with the following results:—

Virus freed from skin particles as follows	Rabbit	Result
By addition of $BaSO_4$ and centrifuging................	A B	2 Small vesicles Negative
By addition of starch and centrifuging................	A B	Definite "take" Negative
By centrifuging only................................	A B	Definite "take" Negative

The fact in this experiment that of two rabbits which received exactly the same material, one was positive, although the take was slight and the other not, probably indicated both a difference in susceptibility of the rabbits and that few organisms passed the filter.

Attempt to clarify by electric current.—Separation of organisms from skin particles was tried by this means. Saline emulsion cannot be used because of course free chlorine is evolved. Hence glycerin and water mixtures were tried,—first, 50 per cent., then 10 per cent., then 5 per cent. With a current of seven milliamperes, passed through 50 per cent. glycerin emulsion, a collection of the organisms (*also of the colloidal skin particles*) took place around the cathode; or possibly there was a killing of the organisms around the positive pole. Three areas on a rabbit inoculated with negative pole fluid, at 10, 20 and 35 minutes of passage of the current respectively, showed good confluent takes, while the 4th area (positive pole fluid withdrawn at 40 minutes) showed *one* good vesicle, and no other sign. At termination of the experiment, the fluid through which the current had passed was distinctly acid to litmus.

Use of electrolytic substances.—Realizing that the clarification by the current was due to the generation in the fluid of minute quantities of electrolytes, an attempt was next made to clarify by introducing electrolytes directly. Cerium chloride and copper sulphate were used, although little hope of success was entertained with the latter. It produced perfect sedimentation after two minutes centrifuging with a water-clear supernatant fluid but inoculation with this un-Berkefelded fluid did not yield a result good enough to justify its further use. Cerium chloride (¼ of a cc. of .025 per cent. solution per cc. of the skin emulsion) left the emulsion still cloudy after three minutes centrifuging at 2040 revolutions, but it proved clear enough to pass the Berkefeld with fifty pounds pressure. Two filters were used, part going through each. These filtrates did not yield a take. UN-Berkefelded control material inoculated intradermally, gave many good vesicles. While the negative result with the Berkefeld V filtrate was distinctly disappointing, this method of clearing should hardly be abandoned without further trial, as adsorption by the filters may have caused the negative result, which might have been different had all the fluid been passed through one filter.

Another attempt at preparing the fluid for the Berkefeld consisted in filtration through alundum cups. This was done primarily to see whether the virus would pass through these relatively coarse filters, as we had then met with no positive results with Berkefeld V. Three experiments were done, all by suction. In one case the rabbit inoculated with the alundum-cup filtrate gave a very fair take, in the second case the take was poor but definite; in the third, the filtrate was passed through Berkefeld V and, when afterward inoculated, gave no result. Each time, B. prodigiosus passed through the cup together with the virus. In fact, the third experiment was done to test the relative coarseness of cups of different grades (there are three) by means of an emulsion of B. prodigiosus. The grading done by the firm manufacturing these cups proved untrustworthy from the filtration standpoint. Thus, of 2 cups of the same grade, one held back a considerably larger percentage of bacilli than the other. Cups graded alike differ in weight, and in apparent density to the naked eye. Poured plates from filtrate through cup RA 84 supposed to be denser than RA 360, representing dilutions of 1-10, 1-1,000, 1-100,000 all showed prodigiosus; plates from RA 360 filtrate showed growth except in 1-100,000. RA 98 gave growth in all 3 dilutions; it was supposed to be the coarsest. When the filtrate from both cups (Ra 84 and RA 360) was pooled and a rabbit used, no take resulted.

Concentration of filtrate in Vacuo.—This can be done over sulphuric acid. Phosphorus pentoxide was used in 2 cases but possesses no advantage over sulphuric acid. A mixture of yellow wax and paraffin was used for sealing the dessicator. A fair degree of vacuum was easy to obtain, one corresponding to 745 mm. of mercury, but leakage may occur. The best make of dessicator must be employed, preferably one in which the sulphuric acid can be placed above the fluid to be dried (Hempel's style). With a good vacuum a great concentration or even evaporation to dryness takes place over night, unless a large quantity of filtrate is used; about 14 cc. in a petri dish became dry.

Analysis of Experiments

Fifteen Berkefeld V filtrates were inoculated on to the skin of 28 rabbits; four definitely positive results (14%), one probably positive, one somewhat suspicious and two very questionable "takes" were obtained.

Suction, and positive pressure up to fifty pounds were tried. Suction was accomplished by a Censo-Nelson vacuum pump.

Filtrates giving positive results.—Of the four filtrates yielding certain takes, two represented virus from one calf, and the other two represented virus from each of two other calves.

Character of the "take" resulting from inoculation with filtrate.—Only one take was obtained by rubbing filtrates into the skin. Other successful inoculations were intradermal, which method allows of 5 or 6 cc. being inoculated upon one rabbit. We, however, inoculated from 1½ to 3 cc. of each filtrate intradermally per rabbit, sometimes using other areas on the same animal to test rubbing-in of the same fluid (1 cc).

The reactions yielded by filtrates differed somewhat in different rabbits, and differed considerably from those of a good "take" obtained with unfiltered virus. The filtrate reactions consisted of inflamed areas raised little or not at all above the surface, but usually fairly well circumscribed. When excised, these areas were found to be hard nodules. In one or two cases when the areas were not circumscribed, it was doubted whether the few inflamed spots did constitute a "take". Both in these cases and in ones thought to be positive, the inflamed areas developed at the proper time as indicated by the incubation. To prove that these constituted a "take", they were excised, ground with sand, a saline suspension made, and—after clearing sufficiently by centrifuging—inoculated into one or more rabbits. The resulting nodules were more numerous, larger and more definite in these rabbits representing the second generation, and developed at the proper time (third or fourth day); in the first generation the nodules were some to many times fewer than were the spots injected. In one second generation rabbit the nodules were extremely large and much inflamd; in smears stained by Gram no bacteria were found.

The atypical appearance of the "takes" with filtered virus was believed to be due to the high dilution resulting from adsorption by the filter. To test this, dilutions were made, running from 1:300 to 1:10,000 of the virus as regularly prepared, that is, .4 gram of pulp to 4.6 cc. of 50 per cent. glycerin solution containing 1 per cent. phenol, or 1:3,450 to 1:115,000 of the pulp itself. These dilutions were inoculated both intradermally and by rubbing in.

The dilutions of 1:1,000; 1:3,000; 1:5,000 and 1:10,000 showed no definite takes on the rubbed-in areas, but showed the discrete inflamed nodules, not projecting, which resembled takes from filtrates, although the latter were less regularly circumscribed.

Positive Filtrate No. 1 (Calf virus 237; Jan. 20, 1921).—Dilution of the virus, by which is always meant total dilution of the pulp, was 1:24 sand-ground 5 minutes; cleared for filtration by centrifuging; Berkefelded with 25 pounds pressure; inoculated on to one rabbit, both intradermally (1½ cc.) and by rubbing-in very thoroughly with the finger (1 cc.—all that skin-area would absorb). As both of these areas were on one side of the animal, it cannot be said with certainty that the take was limited to the rubbed-in area, but—contrary to expectation—in this case the preponderant take was there. On 4th, 5th and 6th days, areas regarded as distinctly suspicious were present; on the 6th day these nodules were excised for reinoculation, which gave an unquestionable take. After most of the diluted virus had gone through under 50 pounds pressure, the small remainder was diluted so that it represented a dilution of 1:48, and was passed through the same filter by suction, the surface being brushed constantly with a rather stiff brush to prevent clogging. The rabbit inoculated with this second filtrate gave a questionable take. Reinoculation was not done.

Positive Filtrate No. 2 (Calf virus 200; Jan. 25th, 1921).—Dilution of virus 1:26; sand-ground five minutes; cleared for filtration by centrifuging; divided into two portions, A and B, both passing through the same filter; A under twenty-five pounds and then B under fifty pounds pressure. One rabbit was inoculated with each filtered portion. The twenty-five pound-pressure rabbit remained perfectly negative until the 11th day when there was one small suspicious area, but two days later this area no longer appeared suspicious. The fifty pound rabbit on the 4th day showed definite nodules on the intradermal area and was considered positive. These were excised and ground with sand as usual, for reinoculation, and the result was unquestionably positive. The rubbed-in area was negative.

Positive Filtrate No. 3 (Calf virus 196; March 29th, 1921). Dilution of the virus to a smaller degree than in the other experiments that proved positive; sand ground five minutes; cleared by centrifuging and Berkefelded with fifty pounds pressure; inoculated intradermally into two rabbits; each receiving 1½ cc. On the sixth day one rabbit showed a suspicious take, which on the seventh day was regarded as positive. Inoculation of excised areas into three other rabbits gave positive results. The other animal was negative, except that on the fourteenth day very questionable small areas were noticed.

Positive Filtrate No. 4 (Calf virus 196, April 6th, 1921). Dilution of virus 1 to 48; not sand-ground; cleared for filtration by centrifuging. Berkefelded under about forty pounds pressure; inoculated intradermally into two rabbits, each receiving 1½ cc. On the fifth day one rabbit was considered probably positive; on the seventh day certainly positive. Reinoculation gave an undoubted take. The companion rabbit remained negative. It was watched for fourteen days.

Two of the above four filtrations were done with fifty pounds positive pressure, one with somewhat under that (fifty pounds were aimed at, but the fluid passed through quickly before that amount was reached), and one with twenty-five pounds. Two of these filtrates represented a dilution of, practically, 1:25; one a dilution of 1:48; and the fourth, probably a much stronger concentration than any of the others. The dilution in this instance was unknown because the virus used was the sediment packed down by the centrifuging when dealing with a 1:48 dilution. This sediment (the supernatant fluid from which was separately Berkefelded for inoculating another rabbit with negative result) consisted of finely divided skin and sand-particles. It was washed with only 4 cc. of saline solution, re-centrifuged, and Berkefelded.

Filtrates giving questionable results.—The rabbits classified as probably positive (also that regarded as somewhat suspicious,—see below) received filtrates obtained by suction.

The probably positive filtrate represented a dilution of about 1:40; was sand-ground, centrifuged, Berkefelded and evaporated slightly in vacuo—about ⅕ to ¼ of its volume. The one rabbit inoculated received 1½ cc. intradermally (also a small amount rubbed in) on each side; 4th day negative; 6th day, few small but distinct vesicles on right side (excised); 7th day, three more very small nodules had appeared. The transmission experiment was not successful (except for 2 very small questionable nodules on one of the two rabbits) but this is more probably attributable to a defect in carrying it out (no sand-grinding was done) than to the nodules not having represented vaccinia ones.

The filtrate which gave a suspicious take represented a dilution of 1:33; was sand-ground, centrifuged, Berkefelded by suction, and thereafter centrifuged 1½ hours. The portion from the bottom of the tubes was inoculated in two rabbits. One was negative for seven days, on the eighth was somewhat suspicious; on the 10th negative. The companion rabbit was negative. The experiment included two other rabbits one of which gave a questionable take. This rabbit received the supernatant fluid, after the 1½ hours centrifuging, which was evaporated in vacuo, inadvertently *to dryness;* emulsified with water, and about ½ cc. injected intradermally. On the third day, and later, there was suspicion of a reaction.

The fourth animal received the filtrate uncentrifuged and unevaporated, and was negative.

A higher percentage of positive filtrates might have resulted except for such circumstances as these: The clearing necessary in order to prevent clogging, as, for example, filtration through paper pulp or long centrifuging may have robbed the virus of a considerable proportion of its strength. In other cases in which the clearing was imperfectly done, the filter soon clogged, causing, perhaps, the same negative result.

Evidently, unless a method of separating virus from accompanying skin is obtained, filtration to free the virus from other organisms is not practical, except for obtaining small amounts of material for culture experiments.

LABORATORY OBSERVATIONS ON THE VIRUS OF THE MALIGNANT DISEASE RECENTLY EPIZOOTIC AMONG POULTRY

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Charles Krumwiede, Herman Gerber and Dorothy J. Provost

When, in September, 1924, an epizootic of a malignant infectious disease devolped among the market poultry in New York City, Dr. Aaron Silkman, Chief Veterinarian of the Department,[1] requested the cooperation of the Bureau of Laboratories to investigate at least two aspects: first, the etiology of the disease; second, the possibility of harm to man. The following report gives the experimental data obtained.

The clinical and postmortem picture of the disease gave every indication that it was septicemic in character. The hemorrhagic lesions suggested the possibility of an infection by one of the members of the pestis group. Chickens were obtained and examined bacteriologically. The chickens used for this purpose were either killed when very ill or examined promptly after death, so as to eliminate postmortem invasion of the blood. Eighteen chickens were thus examined. Direct smears of the blood were negative. Cultures were made from the blood and liver on the various laboratory media, especially on blood-agar. In the majority of instances a very few colonies developed. A study of these indicated that some were most probably contaminations obtained in the process of autopsy and culture. The bacterial varieties more frequently encountered in the cultures were a Gram-positive coccus (4 chickens), a Gram-positive diphtheroid (7 chickens), and a small, slender, Gram-negative bacillus with a slight tendency to polar staining (5 chickens). The former two varieties, when found, were very few in number and were so presumably contaminations from the skin and feathers that they were held but not further studied, except that a chicken was injected with 1 cc of a broth culture of the coccus, without effect. The last variety, the Gram-negative bacillus, seemed the most likely infecting agent, as the colonies per agar plate ranged from 2 to 20. This variety caused hemolysis of the blood.

Broth cultures of this bacillus were injected subcutaneously or intraperitoneally into five normal chickens without effect. The maximum dose was 2 cc. These negative results indicated that none of the bacterial varieties was the causative agent of the disease and no further study of these cultures was made. Cultures of the intestinal contents had also yielded nothing distinctive.

The failure to demonstrate the existence of a bacterial infection of the blood in a disease so distinctly septicemic in character was seemingly conclusive and turned our attention naturally to the probability of a filtrable virus.

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[1] We are indebted to Dr. L. D. Krohn, who carried out the field investigations and supplied us with the infected and normal fowls for this work.

FILTRATION EXPERIMENTS

Blood was collected, therefore, from chickens, killed during the late stages of the disease. This blood was defibrinated and after dilution with four volumes of broth was centrifuged to sediment the red cells and filtered through small Berkefeld filters. Filtration was accelerated by suction, using the filter-pump on the water-faucet. The negative pressure obtainable on the faucet used was 15 inches of mercury. The filtration results were controlled in all instances by cultural examinations. In the first filtrations the presence, before filtration, and the absence, after filtration, of the hemolytic bacillus above described, indicated that the filters were satisfactory. This was not considered a sufficiently rigid control. Subsequently each collection of the blood was very heavily contaminated with *B. prodigiosus* and from 1 to 2 cc. of the filtrate was tested and found sterile. This very rigid test excluded the possibility of leakages and also excluded the possibility of "faults" developing in a filter previously found to be efficient.

Seven such filtration experiments were carried out. In most of the experiments one chicken was fed and one was injected subcutaneously with 2 cc of the unfiltered material and one chicken was usually fed and one injected subcutaneously with 2 cc of the filtered material.

The following summarizes the results:

Blood virus unfiltered, fed to 6 chickens; 5 died, 1 immune.

Blood virus filtered, fed to 6 chickens (1 received 6 cc); 6 survived (1 found immune on subsequent test).

Blood virus unfiltered, injected into 6 chickens; 5 died, 1 immune.

Blood virus filtered, injected into 7 chickens; 6 died, 1 immune.

That we were actually dealing with a multiplying virus and not a filtrable toxic substance was corroborated by the passage of the disease from chicken to chicken after filtration, and by passages with intermediate controlled filtration of the blood. All the chickens in these and subsequent experiments, unless otherwise noted, were kept singly in cages to eliminate the possibility of contact infection. Likewise, all inoculated birds were quartered in a different building than that housing the unused supply. In nearly every instance where birds survived an inoculation, they were again injected with a large dose of virus to determine whether the failures were actually due to an immunity.

These experimental data leave no doubt that the epizootic disease in question was due to a filtrable virus, filtrable in the sense that it passed through a Berkefeld filter under the conditions of the experiments.

LOSS OF VIRUS DURING FILTRATION

The experiments also show that the virus will cause the disease when either fed or injected. A comparison of the results of feeding experiments, before and after filtration, indicate (see below) that there was a distinct loss of virus during filtration. No attempt was made to determine the mechanism of this loss, especially as regards parallel experiments with filters of smaller porosities. A comparison of the feeding and injections after filtration, in the light of further data given below, shows that feeding of the virus is successful only when more virus is present than is necessary to cause infection by subcutaneous injection.

Having definitely shown that the disease was due to a filtrable virus, the next point investigated was the virulence of the virus. Dilutions of the defibrinated blood obtained from dying chickens were tested in increasing dilutions. The subcutaneous injection of as little as 0.000,000,001 cc of blood was found sufficient to cause the disease. On feeding, 0.01 cc caused death, but 0.001 cc was followed by survival.

Not only has the virus extraordinary virulence, as shown by the above data, but it also has an equally high invasive capacity. Several experiments showed that the slightest scratch of the skin, followed by a mere application of the blood virus, was sufficient to cause the disease. As will be noted later, the oral discharges are infectious. A chicken dead of the disease was taken and his bill sharply tapped against the skin (bared by laying aside the feathers) of a normal chicken. This was sufficient to cause the disease. A drop of defibrinated blood, dropped into the eye, also caused the disease in two chickens.

The virulence of the virus for other species of fowl was also tested. These results are incomplete, as the actual minimum infecting dose was not reached because of insufficient experimental material. This was due largely to the fact that we were misled by the apparent natural immunity of these species and used too large doses in most instances.

The smallest doses, of those tested, which caused death on subcutaneous injection were:

Pigeon	0.00,000,001 cc.
Turkey	0.00,001 cc.
Ducks	0.5 cc died, 0.1 cc survived
Geese	0.1 cc (one goose survived; one goose died)
Guinea hen	0.0,000,001 cc.

The time of death in these species averaged close to 48 hours after inoculation. That the above deaths were due to the virus was controlled by subinoculation into chickens, with development of the typical disease.

The virulence of the virus for mammals was also tested. Mice injected intraperitoneally with 0.5 cc. of defibrinated blood virus survived. Guinea pigs receiving 1 cc of blood virus intraperitoneally also survived. Rabbits were injected with 1 cc. of virus intravenously without effect. Two monkeys *(Macacuc)* were each injected with 4 cc. of defibrinated blood subcutaneously and also fed with 3 cc. of the virus. They showed no ill effect. These observations indicate that mammals are naturally immune and that the virus is harmless to man.

Some observations on the excretion of the virus from infected chickens were also made. The oral mucous secretion contains the virus. This secretion, when mixed with broth, caused death on subcutaneous injection. The skin of a chicken was slightly abraded and a swab carrying the secretion was applied; this also caused the disease. It was previously noted that tapping the skin of a chicken with the beak of a dead chicken caused the disease. A swab contaminated with the oral mucous was gently applied to the mucous membrane of the mouth of a well chicken. This sufficed to cause the disease.

The feces apparently contain the virus but not in great quantity or regularity. Feces scraped from the cage bottom, on death of a bird, were emulsified in broth and injected into two birds, of which one survived and one died. The bird that survived was not an immune. The difficulty in obtaining feces from caged birds and the possibility of contamination of the cage bottom with oral secretion led us to attempt a better method. A bird was placed in a jar when evidently ill and the dropping collected immediately when observed. The injection of an emulsion of this material caused death. The deaths following the injection of feces were controlled by smear and culture examination to exclude bacterial infection and by subsequent injection of the blood of dead chickens into normal chickens, which caused the typical disease. The amount of virus was apparently not great as the birds injected with feces did not die till close to 72 hours.

NATURAL SPREAD OF DISEASE

Some of our observations are suggestive of the mode of transfer under natural conditions. These are the presence of the virus in the oral secretions in considerable amount; the presence of the virus, though apparently in less amount, in the feces; the production of the disease by contamination of the mucous membranes of the eye or mouth; the extraordinary ease of infection through slight abrasions of the skin. It would seem that oral infection, through contamination of food and drink, with mouth secretions or through the actual injection of the blood or organ virus obtained from dead birds, was a factor. Injuries of the skin by any means, with contamination of the injury, would also be a factor. We were surprised, however, that our attempts to cause contact infection failed. Two chickens, caged with an inoculated bird which died in the cage, did not contract the disease. This cage was bedded with hay. Four birds, similarly exposed in a cage without bedding, also failed to contract the disease. In both these experiments the contacts were left in the presumably contaminated cages for a week or longer. That they were not immune was shown by subsequent inoculation.

Dr. Krohn, in the preceding article,[1] describes the postmortem lesions in detail. The picture in our experiment birds was similar. The hemorrhagic lesions of the stomach and heart were present in practically every one.

The experiment birds developed a very acute form of disease. The great majority of the birds died in from twenty-four to forty-eight hours. With minute amounts of virus, death was delayed to 72 to 96 hours. No chicken which developed symptoms of illness survived. Illness was not observed, however, in many birds. With some the disease developed and death occurred out of laboratory hours. Frequently birds would be observed that appeared apparently well, only to be found dead several hours later. Where evident illness occurred, death usually ensued very quickly.

A considerable number of varieties of chickens as well as mongrels were used in the experiments. No differences in susceptibility were noted under the conditions of our experiments. It should be noted, however, that the majority of the injections were of considerable amounts of virus. We encountered very few immune birds. The great virulence of the virus under laboratory conditions raises an interesting question as to the mechanism of such an immunity.

There was a strong suggestion that the virus increased in virulence in the laboratory passage from chicken to chicken. This was indicated by the tendency to the shortening of the period of life in our later experiment birds. Over fifty virus passages have been made.

DISEASE PROBABLY FOWL PEST

The question naturally has been raised as to the nature of the disease. Dr. Krohn has discussed this from the standpoint of symptomatology and pathology and has drawn a close parallelism with fowl pest. Our laboratory observations on filtrability of the virus, its virulence, invasive capacity and excretion closely parallel those given by Ostertag. Our species pathogenicity experiments are, however, somewhat different in some details. A tentative conclusion is justified that the disease is fowl pest, though a final conclusion would have to rest on a study of the foreign and domestic viruses, under parallel conditions.

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[1] Journ. of The Amer. Vet. Med. Assn., 1925, Vol. LXVII, pp. 146-170.

Besides our preliminary note on the existence of a disease in chickens due to a filtrable virus, which appeared January 31, 1925, only one other report has appeared, as far as we know, on the etiology of the disease. Brunett offered a preliminary report on his work. His bacteriological examinations were negative. He found that the injection of "minute" amounts of blood caused the disease; likewise the feeding of chopped liver of a diseased bird. He concluded that the disease was due to a filtrable virus. This conclusion he based on two filtration experiments, the filter having been previously tested by ascertaining its ability to retain *Pasteurella aviseptica*. He carried the virus through nine generations but he does not state whether these included the filtered material.

SUMMARY

The recent malignant epizootic disease in poultry was due to a filtrable virus. This virus was highly virulent; 0,000,000,001 cc of blood virus, injected subcutaneously, or 0.01 cc of blood virus, given by mouth, caused death. The mere application of virus to slight abrasions of the skin sufficed to cause infection and death. The virus is present in the oral secretions and also in the feces in lesser quantity. Other species of fowl are susceptible to a varying degree. Mammals are immune. The observations recorded strongly indicate that the disease is the European fowl pest.

For bibliography see the original article.

A DROPPING BOTTLE AS AN AID IN MACROSCOPIC SLIDE AGGLUTINATION

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Charles Krumwiede

The macroscopic slide agglutination method has been more or less generally known for years. The value of the method, however, has not been generally appreciated, probably due to the fact that most of the serums employed for indentification purposes have not been sufficiently active to give an immediate or prompt agglutination even in low dilutions. Coca utilized the method in cholera examinations and, to our knowledge, he was the first to publish a description of the method. We have utilized it for some years as a routine in examinations of feces for members of the typhoid-paratyphoid dysentery types and also as an aid in the search for meningococcus carriers. As very potent sera become more generally available its use will undoubtedly spread to most laboratories.

There is one inconvenient feature in the method which we believe to have overcome by the device here reported. In placing on the slide the drops of diluted agglutinating serum and the saline or diluted normal serum for control it has been the custom to utilize the ordinary platinum loop. Where many colonies are to be tested this method is time-consuming and tedious.

The dropping bottle that we have devised to deliver the drops depends upon the use of a capillary delivery tube with a flat end and a rubber diaphragm to force the delivery of the drop.

The delivery tube is prepared from a piece of heavy walled tubing (thermometer type) having an outside diameter of about 4mm. and a bore of about 1mm. This is heated thoroughly in the flame and drawn out slowly. A piece is then cut as shown in the outline sketch (figure 1) and the larger

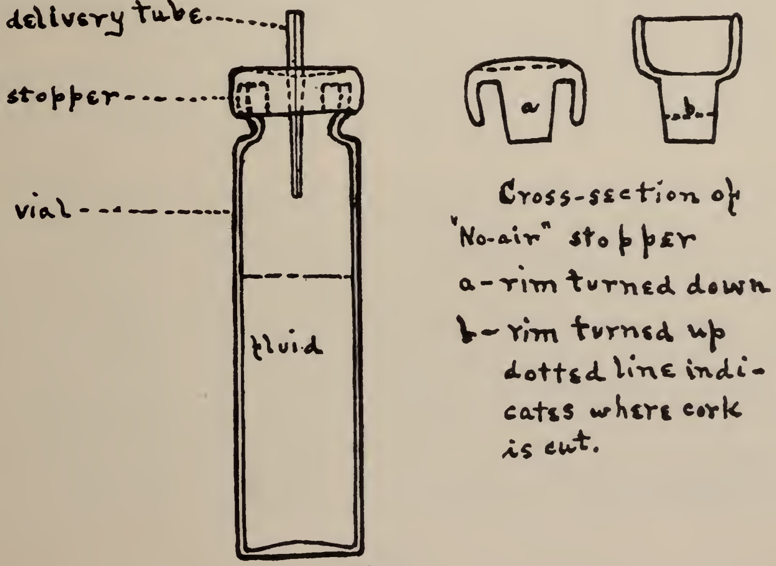
delivery tube
stopper
vial
fluid
a
b
Cross-section of "No-air" stopper
a-rim turned down
b-rim turned up
dotted line indicates where cork is cut.

Fig. 1.
(Reprinted by courtesy of Journal of Immunology.)

end ground flat and smooth on a fine stone. The diameter of the bore is reduced to about the bore of 20 gauge hypodermic needle.

The diaphragm is made from a so-called "no-air" stopper, size No. 2½. The cork end is cut off leaving only sufficient to give stability to the delivery tube. The delivery tube is thrust through the cork.

The bottle employed is the regulation ½ ounce wide mouth homeopathic vial. The neck of the bottle must be wider than the cork portion of the stopper to allow up and down play otherwise there will be no diaphragm action.

The bottles are partly filled with the diluted serums or saline and the drops are placed on the slide by holding the bottle vertical and pressing till the appropriate sized drop is delivered. With very little practice many drops can be delivered in a few seconds. The appropriate pressure to apply soon requires no conscious attention.

The amount of air or air tension in the bottle needs readjustment from time to time. Air can be introduced by inverting the bottle and pulling on the delivery tube. If there is too much air pressure this can be equalized by holding the bottle upright and pressing down the diaphragm which empties the tube and results in equalization of the pressure.

There are other possible applications of this dropping bottle. It may be utilized to deliver drops of saline or other fluids used in the preparation of smears of cultures. In the classrooms it could be utilized to give out suspensions of microörganisms for smear examinations. Other similar uses will probably be found for it.

For bibliography see the original article.

A NEW METHOD OF ADDING CRESOL TO ANTITOXINS AND ANTISERUMS

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Charles Krumwiede and Edwin J. Banzhaf

As is well known, the addition of cresol to antitoxins and other antiserums results in the formation of a precipitate due to the combination of the cresol with the proteins. This precipitate is greatest when all the cresol needed to give the desired concentration is added rapidly. To avoid this more or less voluminous precipitate, various methods have been resorted to. The cresol may be added in a dilute form. This method has the disadvantage of diluting the product to which it is added. Another method is to add the undiluted cresol drop by drop to the product. In this method the serum is commonly placed in an open vessel and beaten with a rod or paddle as the cresol is added to obtain thorough mixing. Similarly, admixture is often accomplished by adding the cresol drop by drop to the large stock bottles, replacing the cork and shaking the container thoroughly after each addition. These methods are not above reproach, as they open the way to contamination. Different producers have devised various methods to limit the exposure to contamination during the addition of the preservative. It is evidently essential that the amount of precipitate formed should be limited as much as possible. In the first place, the precipitate binds up the preservative and thus lessens the amount available throughout the product. How much remains in the serum is not known. Furthermore, the precipitates increase the loss of the product in the process of filtration and add as well to the difficulty of this process.

These considerations raised the question whether we could not find a better method for the addition of cresols to antitoxins and serums. After several preliminary experiments, ether was tried as a solvent. It was found that equal parts of cresol and ether are miscible and make a permanently clear solution. On the addition of such a mixture to antitoxin or serum, the solution floats on the surface causing a slight haze at the point of contact. If shaken immediately after the addition either no precipitate or at most very little is formed. Even the rapid addition of all the solution needed to give a concentration of 0.4% to 0.5% of cresol, causes at most only a slight precipitation; the amount of which is in no way comparable to that resulting from the rapid or even drop by drop addition of the same amount of cresol alone.

With this success in eliminating precipitation when the whole volume of preservative was quickly added, several questions immediately arose: First, what would be the effect of the additional ether when the product is administered? Second, would the product, on aging, precipitate, or in other words would the cresol slowly but eventually cause as much precipitate as if it alone had been added at first? Third, would the loss of ether during filtration and other processes result in objectionable precipitate? Fourth, would the bactericidal action of cresol be decreased by the ether or not? If not, would the additional bactericidal action of the ether be of value? Fifth, would the ether affect the potency of the product?

No untoward clinical effects follow the use of serums containing ether in the concentration noted, even when administered intravenously or intraspinally.

As to the subsequent development of precipitation in antitoxins and serums to which ether-cresol has been added, we can at least say that it is no greater than had cresol been added alone.

The amount of ether lost during the processes of filtration, that is, filtration through paper pulp and through the Berkefeld filter, has not resulted in the formation of a precipitate during the stages of the process or as it finally comes from the Berkefeld filter. The filtered product is crystal clear.

In regard to the fourth question, namely, would the ether lessen the bactericidal action of the cresol or would it leave it unchanged, and if the action of the cresol were unchanged, would the bactericidal action of the ether make the mixture appreciably more active, the disinfectant tests show that the mixture is more efficacious than cresol alone.

On the whole, our experience and that of others who have tried the combined ether-cresol preservative, indicates that the increased bactericidal activity is of decided practical importance, especially in relation to those products which, because of their mode of preparation, cannot be kept sterile.

Experience has shown that the potency of serums and antitoxins is uninfluenced by the ether. Preparations of diphtheria antitoxin, antimeningococcus serum and of antipneumococcus serum have been retested as long as 7 months after addition of the ether cresol. The ether may be added to a toxin-antitoxin mixture without interfering with the balance as the ether is without effect on the more labile toxin element.

Certain theoretical considerations have arisen while collecting the purely practical facts outlined in the foregoing. These points may be mentioned. On mixing the ether and cresol, considerable heat is generated. We have not attempted to determine whether any new compound is formed. If the mixture consists of 25% of ether and 75% cresol, instead of half and half, the addition to serum results in a marked opalescence of the serum although marked precipitation may not occur. Ether tends to reduce the surface tension of the serum. This may possibly be a factor not only in inhibiting precipitation but also a factor in the apparent inhibition of reaction after the intravenous administration of antitoxin. We have not attempted to give a summary of the previous use of ether as a preservative nor of the use of ether in the prevention of anaphylactic shock in experimental animals. The former is generally well known. The latter would involve a discussion which we wish to avoid by simply presenting the practical results obtained without any attempt at explanation.

Summary

A mixture of equal parts of ether and cresol is presented as a new preservative for antitoxins and serums. This mixture is added in amounts necessary to give the required concentration of cresol. The addition of this mixture causes much less precipitate than does cresol alone. Subsequent precipitation is not necessarily limited by the ether, it is never greater than that in products containing cresol alone. The mixture of ether and cresol is more strongly antiseptic than cresol alone. In therapeutic application, the ether is not a disadvantage. In the case of intravenously injected antitoxin, the indication that the ether may under certain circumstances reduce the incidence of adverse reaction warrants further comparative work. Ether may be added to the toxin-antitoxin mixture without disturbing the balance of the mixture.

A COLORIMETRIC REACTION BETWEEN GOLD CHLORIDE AND TOXINS APPARENTLY INDICATIVE OF TOXIC STRENGTH

A Preliminary Report

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Lucy Mishulow and Charles Krumwiede

While we were working on the effects of different agents for detoxifying diphtheria toxin, a report appeared by Osman[1] stating that gold chloride would prevent the toxic reactions usually following the injection of bacteria. This led us to try this agent with diphtheria toxin. In testing the effect of the gold chloride in different concentrations, it was noticed that color reactions occurred. As we were at the same time enagaged in the investigation of the possibility of titrating toxin and antitoxin by physico-chemical means, the idea arose that there might be a quantitative relationship between the reactions with the gold chloride and the strength of the toxin.

Graded mixtures of gold chloride (1 per cent. solution in distilled water) and diphtheria and tetanus toxins were, therefore, tried. Without considering at present the difficulties encountered in determining the optimum conditions, such as concentration of the toxin, etc., the result given in table 1 is representative of a preliminary titration carried out to determine the color range. If we were to utilze the procedure for quantitative comparisons of different toxins obviously an end-point would have to be found. Comparison of the reactions obtained with toxin-broth filtrates and with control-broth of similar constitution suggested that the green-color range was specific for the toxic broth filtrates. A representative titration within the green range is shown in table 2. The point of maximum green coloration was selected as the end-point.

The comparative results with a series of diphtheria and tetanus toxic broth filtrates are given in table 3. The method of titration was as follows:

A 1 per cent. watery solution of gold chloride was pipetted into a series of tubes in gradually increasing amounts (table 2). Then 2 cc. of the proper dilution of toxin were added to each tube. The tubes were shaken to mix the reagents, and incubated at 50°C. Readings were made at the end of forty minutes, and the amount of gold chloride giving the maximum intensity of green color noted. The first toxin in each series was tested on guinea pigs and the M.L.D. was accurately determined. On the basis of this toxin, the probable M.L.D. of the other toxins was calculated on the comparative amounts of gold chloride required to give the greatest intensity of green coloration. Their M.L.D. values were then determined by the guinea-pig test and a startlingly close parallelism resulted with most of the toxins, as may be seen in the table.

The L+ values of some of the toxins were also determined, but these showed no parallelism with the gold chloride values.

Two discrepancies in titrating for the M.L.D. values are shown in table 3. The titration procedure is subject to certain disturbing influences and these were probably the source of the discrepancies.

The main disturbing factors in the method are briefly as follows:

Overlapping of the color-zones sometimes occurs (see table 1). If other colors invade the green zone, the determination of the end-point may become uncertain. Some observations on the use of greater concentrations of sodium chloride in the diluent suggest that the resulting shift of the green zone to a point further removed from the other color zones may eliminate this difficulty.

TABLE 1.

PRELIMINARY TITRATION—TETANUS TOXIN 122

Toxin (Diluted 1:4), c.c. Gold Chloride (1 per cent. watery solution), c.c.	2 0.05	2 0.1	2 0.15	2 0.2	2 0.25	2 0.3
Green coloration	—	—	×	∓	—	—
Purple coloration*	—	—	—	—	∓	—
Blue coloration*	—	—	—	—	—	+
Flocculation*	—	—	—	—	+	+

The tests were incubated in a water bath at 50°C. for thirty minutes.

The diluent = 0.85 per cent sodium chloride in doubly distilled water.

*The sequence of purple and blue colors and the zone of flocculation varies in different lots of toxins.

++ = the most intense coloration; ∓ = very strong coloration; + = strong color; ± = weak color; × = trace; — = no coloration.

TABLE 2.

FINAL TITRATION—TETANUS TOXIN 122

Toxin (diluted 1:4), c.c Gold Chloride (1 per cent solution), c.c..	2 0.16	2 0.17	2 0.18	2 0.19	2 0.20	2 0.205	2 0.21	2 0.22	2 0.224	2 0.226	2 0.228	2 0.23	2 0.232
Green Coloration	×	±	+	+	+	∓	∓	∓	∓	++	++	++	∓

Flocculation occurs when a certain concentration of gold chloride is reached in the mixtures. When the zone of flocculation coincides with the zone of green color, the flocculate carries down the color with it, and accurate readings can not be made. The indications are, however, that the use of a greater concentration of sodium chloride will also eliminate this disturbing factor.

There are certain difficulties to be overcome in this method:

The optimum concentration is not the same with all lots of toxin. In most instances a dilution of 1:3 or 1:4 is satisfactory.

The quantitative range of gold chloride within which the green zone appears is so small that the green coloration may not occur unless close titrations are made. For the same reason great accuracy is required in measuring the reagents. Neutral glassware and doubly-distilled water are essential.

Several scarlatinal toxins have been tested with the gold chloride. A green zone occurred in all of them. The most that we may say at present is that the values calculated by this method and the skin-test-dose values showed sufficient similarities to suggest that the method could be employed for titrations.

TABLE 3.

COMPARISON OF COLORIMETRIC TITRATIONS AND GUINEA PIG TESTS

Toxin	Gold Chloride	Number of M.L.D.'s per cubic Centimeter—Undiluted Toxin	
		Gold chloride test	Guinea-pig test
	*grams**		
Diphtheria 696	0.0042	—†	175
Diphtheria 711C	0.0034	145	150
Diphtheria 717	0.0034	145	150
Diphtheria 718A	0.0046	195	200
Diphtheria 718B	0.0050	220	250
Diphtheria 720A	0.0039	165	175
Diphtheria 721B	0.0039	165	300
Diphtheria 722	0.0084	400	425
Diphtheria 8	0.0068	283	300
Tetanus 118	0.0045	—†	6,800
Tetanus 119	0.0029	4,382	4,500
Tetanus 120	0.00416	6,284	7,000
Tetanus 121	0.00424	6,400	10,000+
Tetanus 122	0.0046	7,743	7,800

*The tests were carried out with a 1 per cent solution of gold chloride in doubly distilled water, and 2 c.c. of toxin diluted 1:2, 1:4, or 1:8 with physiological saline. The results given in the table are calculated on the basis of 1 c.c. of undiluted toxin and the amount of gold chloride used in grams.

†Basis for calculation.

SUMMARY

By means of a colorimetric reaction obtained by adding gold chloride to toxic-broth filtrates we have been able to obtain toxin values which in most instances closely approximate the toxin values obtained by animal tests. Thus far we have not observed a colorimetric end-point which parallels the combining value (toxin-toxoid) of toxins.

We have no data as to the mechanism of the reaction. Whether the reaction is due to a specific toxin-gold chloride combination, or whether some other factor or factors are involved remains to be proven.

Whatever the mechanism of the reaction may be, the results strongly suggest that a practical, gold chloride colorimetric method of standardizing toxic-values of broth filtrates may be developed.

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[1] Osman, M. B.: Jour. Immunol., 1927, xiii, 243.

STUDIES IN INTESTINAL BACTERIOLOGY I

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Agnes Goldman

The object of this investigation was to ascertain the variations of the bacterial flora, both aerobic and anaerobic, in respect to numbers and to species, at certain levels of the small intestine, in health and disease.

A beginning has been made to discover whether such a study may yield any significant results in the investigation of obscure diseases, especially such as have, at various times, been ascribed to focal infections in the intestines or to toxic bacterial products of alimentary tract origin.

An intensive study of one person was made, with the purpose of observing the effect of special feeding on the intestinal and also on the fecal flora, and serologic methods were employed in order to throw further light on the question of an indigenous flora, or of a possible streptococcal focus of infection.

Conclusions

The following conclusions are arrived at from a study of the contents of the human small intestine, chiefly at the jejunal level:

The number of viable bacteria in the contents of a number of normal persons and hospital patients varied within wide limits.

The number of viable bacteria in the contents of diseased persons confined to a light meat-free diet usually appeared to be considerably higher in patients with gastro-intestinal disturbances or with diseases associated with metabolic disturbances or with focal infections of possible gastro-intestinal origin than in patients with diseases apparently unconnected with the alimentary tract.

The flora, in normal and pathologic cases, was usually of mixed bacterial character, forms, with widely varying metabolic requirements, multiplying in the same specimen.

Even when the contents were entirely dominated by one bacterial species, further analysis into cultural and biochemical properties revealed the presence of two or more varieties.

Several specimens from the same person taken under similar conditions usually showed approximate correspondence in bacterial numbers and in the predominant species.

Gram-negative bacilli of the colon group, gamma type streptococci, and gram-positive bacilli of the acidophilus group appeared to be a normal feature at the levels studied.

Spore-bearing aerobes, accompanied by proteolytic activity, appeared to be a normal feature of the intestinal contents.

Anaerobes were absent in four normal specimens but were found in many specimens from pathologic subjects, but not necessarily in all specimens from the same person. Large numbers of spore-bearing aerobes were not as common as in specimens from normal persons.

The presence in considerable numbers of spore-bearing aerobes we attributed in our normal subjects to the presence of meat in the diet.

This investigation has been made possible through the cooperation of Dr. Warren Coleman and Dr. William H. Park, and grew out of the investigations previously made at the suggestion of Dr. Coleman. The method of obtaining intestinal specimens had been developed and reported on by Aranovitch, Coleman and Einhorn. The expenses associated with the part time services of a physician and of the whole time of two laboratory assistants were defrayed by a fund, provided by New York University for Research Investigations in the Department of Medicine of which Dr. Coleman had the disposal. We wish to thank Dr. Park and him for valuable suggestions made to us in the course of the work.

We are also indebted to Dr. Curphey for obtaining the intestinal specimens and to Mrs. Betty Kolchin and Miss Helena Otto for their valuable assistance in the laboratory.

For bibliography see the original article.

STUDIES IN INTESTINAL BACTERIOLOGY II.

The Effect of Special Feeding on the Intestinal Flora

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Agnes Goldman

In the first paper in this series, I discussed the bacterial flora of the small intestine of 4 normal persons and of 20 hospital patients.

One of the female patients W. (arthritis deformans) was particularly ready to take the intestinal tube, and was most conscientious in following directions. She was kept at the hospital for several months, at the request of Dr. Coleman, receiving treatments in the form of bakings, massage and vaccine injection for the arthritic condition.

Altogether 20 intestinal specimens were obtained from her. The methods were the same as those described in the earlier paper. In the work with B. acidophilus we attempted to make use of whey agar plates as recommended by Rettger and Cheplin with but little success.

During much of the time she was receiving B. acidophilus culture (Arlington Chemical Co.), usually 6 teaspoonfuls a day. Later we isolated the strain from one of the bottles, and prepared with it cultures in dextrose broth. The strain isolated corresponded morphologically and culturally to descriptions of B. acidophilus, except for the fact that it did not ferment maltose. We do not know whether this strain never fermented maltose, and therefore did not correspond to the usual definition of B. acidophilus, except for the fact that it did not ferment maltose. We do not know whether this strain never fermented maltose, and therefore did not correspond to the usual definition of B. acidophilus, or whether it had lost this property.

The number of viable bacteria in the broth cultures, which were tested from time to time, varied considerably. The strain was extremely capricious. A fresh 18-hour culture had at least 200,000,000 viable bacteria per c.c., and sometimes several billion.

Table 1 is divided into two parts. In the first part are given the results while the patient was at the hospital on regular hospital diet receiving treatment and showing considerable improvement in her general condition. During most of the time she was getting 6 teaspoonfuls a day either of the commercial preparation called Bacid, or of our laboratory preparation. There is reason to believe that the culture which she was taking from June 20 to July 20, and which was of our own preparation, was largely nonviable, as it was probably kept too long.

All recent experiments agree that such small doses of B. acidophilus, especially if they are not reenforced with lactose feedings, are entirely ineffectual in changing the flora, and we may, therefore, look on this first set of records up to July 6 as observations under normal dietary conditions.

The first 4 specimens, taken over a period of 1 month, show greater variation in bacterial numbers than the earlier specimens. A count of 66,000,000 was far in excess of anything found in the previous specimens of this patient. No reason was found to account for the sudden increase in numbers or the predominance of gram-negative bacilli, which gave typical B. coli-communis sugar reactions, but it is possible that we here have an ex-

STUDY OF INTESTINAL CONTENTS OF W. OVER A PERIOD OF FOUR MONTHS

Date	Nature of Specimen	Feeding	Number of Bacteria per C.c. of* Intestinal Contents or of Fecal Suspensions on			Predominant Bacteria on Aerobic Pour Plates	B. acidophilus Group	Approximate Percentages# of B. acidophilus Group of Total Aerobic Count
			Endo Plates	Blood Veal Plates	Acid Beef Liver Plates			
Summary of Results Under Normal Conditions and After Special Feeding								
5/ 3	Intestinal, approximately 70 to 80″	....	10,200	1,200,000	3,240,000	Streptococci	+	< 1
5/19	Intestinal,† 70″	5/4-5/21: 6 teaspoonfuls of Arlco B. acidophilus culture daily	3,000,000	45,000,000	66,000,000	Colon group	+	< 1
6/ 2	Intestinal, approximately 50 to 60″	....	3,000	4,000	4,000	Streptococci	—	< 1
6/ 7	Intestinal, approximately 50 to 60″	6/3-6/13: 6 teaspoonfuls of Arlco B. acidophilus culture daily	< 20	7,200	2,400	Diplococci	+	< 1
7/ 6	Intestinal, approximately 45 to 50″	6/28-7/7: 150 gm. lactose daily.....	550,000	1,062,000 (80% streptococci)	1,457,000	Streptococci	+	< 1
7/14	Rectal swabbing	7/7-7/13: 100 gm. lactose daily.....	1,500,000	2,000,000 (37% streptococci)	5,300,000 (22% B. acidophilus)	Colon group	++	20
Summary of Intensive Feeding Experiments with B. Acidophilus and Lactose								
8/ 2	Intestinal, 90″	7/19-7/28: 6 teaspoonfus laboratory culture of B. acidophilus freshly grown every 3-4 days	660,000	6,000,000	9,400,000	98% streptococci	++	> 1
8/ 2	Rectal swabbing	7/28 and 7/29: 100 gm. lactose daily; 7/30 and 7/31: 50 gm. lactose daily; 8/1; no lactose	100,000	1,600,000	400,000 (30% B. acidophilus)	Colon group	++	5-10
8/ 5	Intestinal, 100″	8/2: 30 c.c. laboratory culture of B. acidophilus strain fed through 90″ tube; 8/3 and 8/4; no feedings	820,000	1,450,000	1,370,000	Streptococci	+	< 1
8/ 5	Rectal swabbing	....	1,600,000	1,700,000	1,300,000	Colon group	++	4.5
8/11	Intestinal, 100″† Some coiling in stomach, approximate position doubtful	8/5-8/7: no feedings; 8/8: 75 c.c of laboratory culture of B. acidophilus strain fed through 100″ of tube; 8/9, 8/10: no feedings	3,050,000	5,520,000	7.310,000	Streptococci	++	> 1.5
8/15	Intestinal, 80″	8/11: 75 c.c. of laboratory culture of B. acidophilus fed through 100″# of tube plus 100 gm. lactose; 8/12, 8/13, 8/14: 100 gm. lactose daily	< 20	13,400	13,600 (yeast)	Streptococci and sporing gram-positive bacilli	—	< 1
8/15	Rectal swabbing	....	109,200	108,400	268,000	Colon group	—	< 1
9/14	Intestinal, 65 to 70″†	8/22-9/15: 250 c.c. daily of laboratory culture of B. acidophilus strain plus 100 gm. lactose daily........	29,800,000	16,800,000	14,700,000	**Streptococci and** colon group	++	1.5
9/14	Fecal swabbing	....	85,000	125,000	148,000	Colon group	+	< 1

++, present in large numbers or amounts; +, present; —, not present as far as observed.

* The fecal suspensions from rectal swabbings probably represented between 2-10 milligrams of feces per c.c.

† The high counts on these intestinal specimens indicate that a reversed peristalsis may have taken place.

# The approximate percentage of B. acidophilus was estimated by noting the percentage of colonies present on the acid beef-liver plates and by noting the highest dilution of the N/10 acetic broth tubes in which morphologically characteristic bacilli developed.

ample of reversed peristalsis such as later occasionally was found to take place. The next 2 specimens showed an unusually low count. Another sample not included in the table, taken June 14, with an open olive, showed a count of less than 20,000 bacteria per c.c.; thus over a period of about 2 weeks, 3 successive specimens gave evidence of a decided reduction in bacterial numbers. Whether this reduction was related to the patient's generally improved condition or is attributable to the somewhat higher level from which the specimens came, we are not in a position to state. Some coiling of the tube was reported, and it was difficult to estimate the depth of the olive.

On June 28 she started taking lactose, 150 gm. per day until July 7, followed by 100 gm. per day from July 7 to 13. On July 3 a mild diarrhea set in. A specimen taken on July 6, shows a rise in bacterial numbers to over 1,000,000 per c.c.

The last specimen is a rectal swabbing taken at the close of the period of lactose feeding. A suspension in broth or salt solution was made from all swabbings. The amount of material was not weighed, as comparative rather than absolute numbers were desired. Counts on the different plates poured from the same rectal or fecal specimen give indications of the proportions of different bacterial groups. One c.c of suspension represents approximately between 2 and 10 mg. of feces.

A noteworthy point is the fact that all intestinal specimens, except the one with an excessively high count, show a predominance of streptococci. Even the rectal swabbing shows a fairly high percentage (37%) of green-producing streptococci.

We may note another related fact, that in the case of all the high count specimens the plates poured with acid beef-liver medium are by far the most crowded, even in the one with a predominance of gram-negative bacilli. This indicates that the flora of this patient was glycophilic and decidedly aciduric. The feeding of lactose encouraged these aciduric and glycophilic organisms both in the small and large intestine.

On investigating the proportion of B. acidophilus, one finds that, as might be expected with such an aciduric flora, this organism was usually present although in small numbers. It often overgrew all other organisms in the milk tube, and it was frequently isolated from the N/10 acetic acid broth. The approximate percentage of B. acidophilus-like bacilli of the total aerobic plate count has been put down as less than 1% whenever there were too few present to make an estimate, although in many cases the proportion is probably much under 1%.

Since the members of this group were normally present, we should expect the lactose to stimulate their development. From the intestinal specimen of July 6, we now find a few characteristic colonies appearing on the acid beef-liver plates, but on the whole the sugar is much more strongly favorable to the streptococci. The most crowded, however, of the acid beef-liver plates, poured from the rectal swabbing, showed 22% of B. acidophilus colonies. The acid beef-liver plates poured from earlier rectal specimens had no colonies of this type. We may say, at this point, that our search for B. acidophilus was thorough. We looked not only for the fluffy, but also for the smooth edged, types of colony; and we made many isolations and stained films from colonies whenever we were in doubt.

The second section of table 1 is given over to the record of an experiment in feeding a viable B. acidophilus culture through the tube, and then for two weeks administering large quantities per os, together with lactose. Our object in the first case was to avoid the action of the gastric secretion.

We intended to give the patient a certain length of tube to swallow, to take a specimen, and then before removing the tube, to inject some culture into the intestine. The patient was then to have a day of rest, and on the third day we were again to give her the tube to swallow with the purpose of having the olive reach a point below the level attained on the previous occasion. The object was to ascertain, through the intestinal specimen, and through a rectal swabbing, whether B. acidophilus had established itself below the point of injection. We then intended to follow the same method of procedure, administering culture and lactose simultaneously, the lactose, however, being taken by the mouth.

The chief difficulty was in controlling and confirming the point reached by the tube. According to the roentgen-ray reports, there seems to have been some coiling in the stomach on several occasions. Nevertheless had B. acidophilus become established, we might expect it to spread over a considerable region.

Besides pouring the acid beef-liver plates with 3 dilutions we also made dilutions of the specimens in a series of N/10 acetic dextrose broth, using from 0.5 c.c. of 1:10 to 0.1 c.c. of 1:10,000 or 0.1 c.c. of 1:100,000 dilution. Sometimes these tubes gave evidence of a growth which on the stained film morphologically resembled B. acidophilus, when no typical colonies could be found on the acid beef-liver plate. Often the bacilli in the N/10 acetic broth were nonviable—development was apparently initiated and then inhibited.

In obtaining the percentage of B. acidophilus, we made use both of the acid beef-liver plates and the N/10 acetic broth tubes. If morphologically characteristic organisms were found on the stained film from a N/10 acetic tube made up with 0.1 c.c. of a 1:1,000 dilution of the original material, while none was present in a tube containing 0.1 c.c. of a 1:10,000 dilution, we considered that between 10,000 and 100,000 B. acidophili were present per c.c. We then compared this number with the highest count on a plate having no B. acidophilus colonies. While it might be objected that many bacteria of different species which are too feeble to grow on a plate might develop in a liquid medium, and that therefore we were estimating our percentage of B. acidophilus too high, it is also true that N/10 acetic broth even with 2% dextrose is a severe medium, and that therefore a tube must be fairly heavily inoculated for growth to take place.

The two specimens, intestinal and rectal, taken on August 2, followed a week of B. acidophilus feeding (6 teaspoonfuls a day) and 3 days of lactose feeding. In the intestinal specimen over 1% and in the rectal specimen from 5 to 10%, of B. acidophilus was present. After stopping the lactose, and feeding 30 c.c. of culture directly through the tube, there was an apparent drop of B. acidophilus content both in the small intestine and in the feces. After feeding 75 c.c. of culture, there was apparently a slight rise of B. acidophilus in the intestinal content, but a further drop in the rectal content. August 15 another 75 c.c. of culture was injected through the tube, and on this and the following 3 days 100 gm. of lactose was taken. Neither the intestinal nor rectal specimens showed any B. acidophilus. Moreover, in none of the specimens was there a reduction of the amount of gas in the dextrose broth fermentation tubes. From this experiment, we conclude that a single injection of a concentrated B. acidophilus culture directly into the small intestinal tract is of no value in establishing the organism. The results might be different if the tube were retained long enough for several injections to be made. We wish, however, to call attention to the presence of free spores in the sediment of the dextrose fermentation tube (the record of observations on this medium is not included in the table), and to the predominance of yeast on the acid beef-liver plates of the intestinal specimen of August 15. Kendall observed that when animals are fed for some time on carbohydrates, gram-positive spore-bearing bacilli which slowly liquefy gelatin appear as a kind of intermediate adaptive flora. The spores in the sugar tube and the yeast seem to us to represent a type of intermediate flora appearing in response to a few days of lactose feeding.

Finally, we fed our patient for 2 weeks daily with 250 c.c. of a laboratory culture in dextrose broth of B. acidophilus, together with 100 gm. of lactose. We then had every reason to expect an implantation. After this period the intestinal contents showed between 1 and 10% of B. acidophilus-like bacilli in the N/10 acetic broth, and no typical colonies on the acid beef-liver plates. We again note the great increase in total numbers observed in a single previous specimen. From a fecal swab we made an estimate of less than 1%. This gave lower results than had been given by a previous feeding with lactose alone. A fecal swabbing taken 4 days after the feedings were stopped showed about 5% of B. acidophilus colonies.

The question naturally arises whether the strain used by us, derived from a commercial bottle, was adapted to implantation. We stated at the beginning that this strain had lost, if it ever had possessed, the faculty of fermenting maltose, which is supposed to be characteristic of B. acidophilus. In this connection we wish to state that every strain resembling B. acidophilus isolated either from the acid beef-liver plates, milk or acetic broth tubes, and tried on maltose, invariably fermented this sugar, some strains more slowly than others. From this result we must conclude that there never was any implantation of the strain administered, and that fluctuations in the percentage of B. acidophilus were entirely due to lactose feedings or to some other fortuitous factor. Work carried on in our laboratory by Miss Otto indicated that with several other strains also true implantation did not take place.

Experiment with Sterile Food

The patient left the hospital and returned to her home on Oct. 1, 1922. November 20, she came late in the evening to the Willard Parker Hospital, where she was under our direct observation. That night she took a cathartic. From the following morning she was served with food that was as nearly sterile as possible. She received sterile water, boiled milk, oranges, boiled eggs, and thoroughly toasted bread. Her dishes were kept apart and boiled. She washed her hands thoroughly and gargled with a disinfectant before eating. The only nonsterile food she received was 100 c.c. of a laboratory culture of the same B. acidophilus strain which she had previously taken. On Nov. 22 the tube was passed, and on the morning of the 23rd a specimen was taken. During this entire period her food was controlled.

The roentgenogram giving the position of the tube, of which 75″ were swallowed, showed that no coils were present.

Great difficulty was encountered in aspirating any material. It was finally necessary to inject about 20 c.c of salt solution, followed by some air, to expel it from the tube, and then after waiting from 5 to 10 minutes, to aspirate again. By doing this twice, enough material for cultivation was obtained.

With the exception of 3 surface colonies of staphylococci regarded as contaminations, three plates, each of which contained 0.05 c.c. of the original material, were entirely sterile. So also was the dextrose broth fermentation tube, and the N/20 acetic dextrose broth. In the sugar-free broth fermentation tube we found a small diplococcus. A similar organism and a few small gram-positive bacilli were found in the stained film from the lowest dilution tube of the cooked meat medium. In the milk, which slowly coagulated, we found a minute, long chain streptococcus, 15 to 18 faintly gram-positive individuals constituting a chain. On cultivation, this strain formed minute colonies of the gamma type on a blood veal plate.

A first glance at the result of this experiment inclines one to the opinion that the bacterial flora of the small intestine may be determined by the bacterial features of the ingesta. There are, however, several reasons why such a conclusion seems unwarranted.

First, we must remember that several months had passed since the patient was discharged from the hospital, and that her general condition was much improved over what it had been during the first months after entering the hospital. She had not been taking any lactose, which, as we have seen, had a stimulating effect on the normal intestinal flora. Some recent experiments, not recorded here, have given confirmatory results, that is, they indicate a great natural reduction in the numbers of the intestinal flora.

Another important point is that in our effort to give her sterile food, we simplified her diet greatly. Except for a little bread, the food was liquid or semisolid, and for the most part easily digestible. Probably a large part of it was absorbed in the upper levels of the small intestine, and what remained passed rapidly into the colon without offering a nidus for the breeding of bacteria, such as would be afforded by masses of undigested meat or of carbohydrates.

The fact that the only organism successfully recovered was a delicately growing streptococcus, a member of the group most characteristic of the intestinal contents of this patient, would seem to suggest that here we have a representative of what, for this person, constitutes a part of an obligate indigenous flora. In the later experiments, just referred to, it was again found that the only recoverable organisms in two apparently almost sterile specimens were streptococci, this time of the alpha type, developing best in the milk tube.

Finally, the fact that the large numbers of viable acidophilus bacilli ingested were not recovered denotes that they passed directly out of the small intestine, without even temporarily establishing themselves.

Thus, it would seem to us that while bacteria introduced with the food or saliva may temporarily multiply in the interior of undigested masses of food, the bacterial numbers will rather depend on the amount and the nature of the diet than on its microbal constituents, while the basic flora, like that of the upper respiratory passages, will maintain itself through considerable environmental variations. It seems possible, however, that the rapidity with which the bacteria are eliminated may depend on the substratum on which they are introduced. Those in a liquid medium might be rapidly disposed of through the action of peristalsis, while those embedded in a more solid substance might multiply, especially in the presence of sluggish peristalsis, until they temporarily overshadowed or at least modified the picture of the permanent flora.

A similar series of experiments, over a protracted period, should of course be carried out on one or more normal persons, in order to determine whether a certain bacterial type is always predominant in spite of a variety of environmental changes.

SUMMARY

The strain of B. acidophilus fed in large quantities to one patient could not be implanted in the intestine. Lactose feeding was more effective in increasing the number of B. acidophilus. It seemed most effective, however, in increasing the number of streptococci.

Large numbers of viable B. acidophilus introduced directly into the small intestine were rapidly eliminated.

After a diet sterile except for a broth culture of B. acidophilus the intestinal content at a depth of 75″ was almost sterile, streptococci being the only recoverable organisms in a few tubes of liquid medium.

It is suggested that streptococci may represent the indigenous bacterial flora of this person at the level tested.

For bibliography see the original article.

STUDIES IN INTESTINAL BACTERIOLOGY III

A Study of Nonhemolytic Streptococci from Human Intestinal Tract.

Published in The Journal of Infectious Diseases, 1924, Vol. XXXIV, pp. 509-515

Agnes Goldman

Investigations on the antigenic relationships of the Streptococcus viridans group from human throats, have demonstrated its heterogeneous character. From the same person at a given time, two or more culturally similar but antigenically distinct strains may be isolated.

It seemed of interest, therefore, to examine the streptococci isolated from the intestines of one person at the same and at different times. Opportunity for such a study was afforded by material obtained through the Einhorn tube from patient W. in the manner described in the previous papers of this series.

The existence of similar immunologic reactions in two or more strains isolated at different times might throw some light on the question of a native or permanent flora in the small intestine. When the patient, as was the case with our subject, is suffering from arthritis deformans, often ascribed to focal streptococcal infection, the frequent reappearance of the same strain might indicate a possible relationship of the strain to the disease.

N. and J. Mutch state that there is a great increase in the absolute and relative numbers, and in the varieties of intestinal nonhemolytic streptococci in certain conditions of disease. Holman observes that focal areas of streptococcal infection often contain more than one type of streptococcus. There is also evidence that when green-producing streptococci appear in the throat associated with colds or influenza, a greater variety of strains is present than is found in normal throats.

The varieties isolated ranged from strains growing luxuriantly to those growing sparsely on ordinary dextrose agar, although none seemed as delicate as two strains obtained from a nasopharyngeal swabbing of the same patient. Some were of the gamma type; others were of the alpha type and produced a vivid green zone, followed by marked clearing after standing at room temperature, but most strains occupied an intermediate position, only slightly discoloring the blood, and developing a small hemolytic zone. Isolations were made from different mediums as indicated in table 1, which gives immunologic relationships, and sugar reactions of a number of strains derived from 9 intestinal and 1 nasopharyngeal specimen.

If one first examines the sugar reactions, it is seen that the intestinal streptococci fall into several groups. As might be expected, Strep. fecalis was isolated on several different occasions. Strep. equinus reactions were given by all strains tried on sugars from examination 4 (strains 11-14). They were originally rather delicately growing organisms. Strep. mitis, which, according to Holman, is the most common of the nonhemolytic strains, was recovered from 4, possibly from 5, specimens. Occasionally a sugar reaction was so faint (∓) that without repeating the test it would be difficult to classify the strain. Strep. ignavus was twice isolated. Strep.

TABLE 1

AGGLUTINATION AND SUGAR REACTIONS OF STRAINS OF STREPTOCOCCI ISOLATED FROM INTESTINAL SPECIMENS AND NASOPHARYNX OF W.

Cultures			Agglutination Reactions with Serum of Rabbit Immunized with						Sugar Fermentations					Classification According to Holman
			Strain 1		Strain 11		Strain 9							
No of Strain	No. of Specimen	Medium	Titer	Control Serum 1:100	Titer	Control Serum 1:100	Titer	Control Serum 1:50	Dextrose	Lactose	Mannite	Saccharose	Salicin	
1	1	Blood	1,600	0	0	0								
2	1	Blood	0	400	400	100	600	50	++	++	±	++	++	Mitis or fecalis
3	1	Cooked Meat	200	0	0	0	0	0	++	++	++	++	++	Fecalis
4	1	Endo	200	0	0	0	0	0	++	++	++	++	++	Fecalis
5	1	Endo	1,600	0	0	0								
6	1	Acid beef liver	800	0	0	0	0	0	++	++	—	++	++	Mitis
7	2	Blood	0	0	0	0								
8	2	Blood	0	0	0	0	0	0	±	—	—	—	—	Ignavus ?
9	3	Blood	0	0	1,400	100	600	0	++	±	—	±	++	Equinus or mitis
10	3	Blood	0	0	0	0								
11	4	Blood	0	0	1,400	0	0	0	++	—	—	++	++	Equinus
12	4	Blood	100	0	100	0								
13	4	Blood	0	0	1,400	0	0	0	++	—	—	++	++	Equinus
14	4	Blood	0	0	200	0	100	0	++	—	—	++	++	Equinus
15	5	Cooked Meat	0	0	0	0	0	0	++	++	++	+	++	Fecalis
16	10	Acid beef liver	1,600	100	0	0	0	0	++	++	—	++	++	Mitis
17	10	Acid beef liver	0	0	0	0	...	...	±	—	—	++	—	Ignavus
18	15	Blood	200	0	0	0	600	0	++	++	—	±	++	Mitis
19	15	Blood	0	0	400	0	0	0	++	++	++	++	++	Fecalis
20	19	Blood	.....	...	0	0	0	0	++	++	—	++	—	Salivarius
21	19	Whey	.....	...	.....	...	0	0	++	++	—	++	++	Mitis
22	19	Acid beef liver	.....	...	.....	...	0	0	++	++	—	++	±	Salivarius or mitis
23	25	....	.....	...	.....	...	0	0	++	++	++	++	++	Fecalis
24	Nasopharynx	....	.....	0	0	0	0	0	++	++	—	+	—	Salivarius
25	Nasopharynx	....	1,600	0	0	0	0	50	++	++	++	++	++	Fecalis

The following symbols are used to indicate sugar reactions: ++, marked fermentation; +, faint fermentation; ±, doubtful fermentation; —, no fermentation;
aThe titer of a serum for any strain is the highest dilution showing complete or almost complete agglutination.

salivarius was isolated from one intestinal specimen only. Particular reference is made to this as it was unique.

The lower dilution blood plates from the original specimen showed a mixed flora, but the highest dilution plate was exclusively seeded with colonies of streptococci, which were eventually found to constitute over 98% of the total flora. One further analysis, the streptococci from this sample fell into two groups: one, represented by strain 21, formed short chains in broth, and moderately green-producing colonies on blood agar; the other, represented by strain 20 from a blood plate and strain 22 from an acid beef-liver plate, was found as a lance-shaped diplococcus that only after several transplantations began to form any chains in broth. The colonies on blood agar were minute, surrounded by a relatively wide vivid green zone. They resembled much more nearly the characteristic alpha type found in the throats of patients with cold and influenza. It is, therefore, noteworthy that they gave the sugar reactions of Strep. salivarius. This group is supposed to be found rarely in the intestine, but is often associated with focal areas in the nasopharynx. Two strains from this source (strains 24 and 25) gave the sugar reactions of Strep. salivarius, although they continued, even after long cultivation, to grow much more delicately than the strains 20 and 22 from specimen 19. The single strain of Strep, salivarius isolated by Holman from the feces was a long chain variety, in this respect differing from ours. We tried repeatedly to isolate a similar type from subsequent specimens, but were not able to recover a strain culturally resembling the diplococcoid form of strains 20 and 22. As is well known, Poynton and Paine found a diplococcus which they thought was directly associated with rheumatoid arthritic conditions.

If one now turns to the cross agglutinations which were carried out with serums from rabbits immunized against strains isolated from the first, third and fourth intestinal specimens, one finds that serum of strain 1 partially agglutinated all of the strains from the same specimen, except strain 2. It also slightly agglutinated a few strains from later specimens. Some of the strains partially agglutinated by the serum may be more closely related to the immunizing strain than appears from the result. It is probable that the agglutination in higher dilution of the immunizing strain may be due to its constant transplantation during the period of injection. Nevertheless, there are few strains that are completely agglutinated by the serums even at the dilution of 1:100. The only "fishing" completely agglutinated by serum of strain 1 was a streptococcus fecalis of nasopharyngeal origin. The two strains strongly resembled one another in morphologic and cultural characteristics. Unfortunately absorption tests could not be made because strain 1 died out during the course of the work. The relationship is suggestive, but no definite conclusions can be drawn without the confirmatory test.

Serum of strain 11 strongly agglutinated one strain from the same specimen and none from any other specimen. Serum of strain 9 was not tried on any strains from the same specimen. It strongly agglutinated one strain from specimen 15, i. e. strain 18. In order further to investigate the relationship between the two strains, an absorption test was carried out. It was found that strain 18 completely absorbed the specific agglutinins for strain 9. A rabbit was then immunized with strain 18 and reciprocal agglutination and absorption tests were made. It then appeared that strain 9 absorbed all the specific agglutinins for 18. Thus by the most delicate test at our disposal the identity of the two strains was established.

The data of the absorption tests are given in table 2. The method followed was that used in our serologic study of alpha streptococci from the throat.

TABLE 2.

AGGLUTINATION BY SERUMS 9 AND 18 BEFORE AND AFTER CROSS ABSORPTION

	Strain 9	Strain 18
Serum 9	1,000	1,000
Serum 9 treated with strain 9	0	0
Serum 9 treated with strain 18	0	0
Serum 18	1,000	1,000
Serum 18 treated with strain 9	0	0
Serum 18 treated with strain 18	0	0

We conclude from this set of experiments that there must be present in the upper intestinal tract of W. at different times a considerable variety of streptococcal types. Nevertheless, the fact that we have isolated, at intervals of 2 months, 2 strains apparently identical, may indicate either that there is a flora which is more or less permanently established in the intestinal tract or that there is a focus at some other point from which these organisms are introduced into the alimentary canal.

While it is of course quite possible that one streptococcal strain among many in the tonsils, teeth or intestinal tract may be directly responsible for the symptoms of disease, the mere presence of an unusual number and variety of nonhemolytic streptococci hardly justifies the assumption of a causative inter-relationship of any one or all of them to the pathologic condition, especially in view of the fact that these bacteria are common secondary invading organisms. N. and J. Mutch suggest that "a negative phase in the immunity reactions of the body, determined by increased bacterial activity at one focus, affords bacteria of allied race, opportunity of gaining simultaneously advantage over the cells of distant tissues among which they live."

In view however, of the recognized adaptability to mucosal surfaces of the nonhemolytic streptococci, the establishment of members of this group in the small intestine does not necessarily seem to imply the existence of a pathologic flora, any more than does the extreme predominance of the colon group at lower levels of the intestine. It seems plausible to regard the streptococci, when present in large numbers, as indications of deranged intestinal functioning or of excessive amounts of carbohydrate in the diet rather than as evidence of the existence of a primary focus of infection with production of toxic substances or with tissue invasion. Lactose feeding, we found, stimulated this group, and it has been affirmed that the presence of carbohydrate associated with loci of stagnation is the most potent factor in establishing its predominance just as undigested proteins are supposed to favor the proteolytic organisms. It seems probable that an adaptation of particular bacterial types to the metabolic conditions within the tract of each person may gradually develop, until certain individual strains become indigenous.

Several strains of intestinal streptococci were tested for direct agglutination by the serum of the patient W. using normal serum as control.

As the patient had some months earlier received an autogenous vaccine of intestinal streptococci, positive reactions were expected (table 3).

TABLE 3.

AGGLUTINATION TESTS BY WALSH AND GOLDMAN SERUMS OF STRAINS OF INTESTINAL STREPTOCOCCI

Number of Strain	Serum	Titer
20	W	3,200
	G	1,600
11	W	100
	G	100
18	W	3,200
	G	400
9	W	3,200
	G	1,600
4	W	100
	G	100
25	W	0
	G	0
Burke	W	1,600
	G	800

Agglutinations were carried out on 2 separate days; the first set was carried through a 1:100 serum dilution. The second set was carried out with 1:100 as the lowest serum dilution.

In the first test, we used 1:100 as the highest dilution, but when it was found that in all lower dilutions a prezone developed, we repeated the test with higher dilutions, using in the case of the patient some serum that had been held over for 3 days.

It was found that both the patient's and the control serum agglutinated most of the strains in high dilution. The patient's serum was the more potent, but the difference seems of little significance. This was the case not only when the serum was tested with autogenous strains, but also when tested with a strain isolated from the specimen of another subject (B). The test with the streptococcal strain of naso-pharyngeal origin was negative. Unfortunately it was not possible to test it with as high a dilution of serum as was used for the other strains.

These results prove how misleading the mere fact of a high titer agglutination may be. They also indicate that since human serum appears to have developed a high antibody content against intestinal streptococci, these organisms must be regarded, as indeed the flora of the feces would lead us to suppose, as normal inhabitants of the intestinal tract.

SUMMARY

The same strain of nonhemolytic streptococcus, identified by agglutinin absorption methods, was twice recovered from the intestinal contents of one patient, at an interval of two months.

The persistence of this strain may indicate a well adapted flora, native to the intestinal tract of the person from whom the strain was isolated or a focus at a higher level from which the strain was at intervals disseminated.

Normal human serum appears to contain a high antibody content of group agglutinins for some nonautogenous streptococci from the small intestine.

For bibliography see the original article.

HYDROGEN ION CONCENTRATION.

A brief and simple discussion of the meaning of pH and a modification of Medalia's method for its determination in the phenol red range.

Jacob Weinberg

Introduction

In the last ten years the "hydrogen ion concentration," the measurement of acidity by the so-called pH, has constantly been gaining in popularity. It is now being extensively applied in numerous industries and especially in the various branches of the biological science. Quite frequently, therefore, such tests have to be performed by workers who cannot afford to go into the details of the theoretical considerations of the pH, for these as treated in the modern works on physical chemistry involve higher mathematics and principles of a complicated nature. An elementary simple and brief discussion of hydrogen ion determination will in many cases be helpful. For this reason before commencing the treatment of the problem an attempt will be made to touch upon some fundamental points related to hydrogen ion concentration. One of these is the definition of an acid and a base.

An acid is defined as a substance which when in solution

1. Turns blue litmus red;
2. Gives a sour taste;
3. And reacting with a base yields a salt:

   or
4. Dissociates into its components, one of which is hydrogen ion.

All of these definitions, though stating the true qualities of an acid, are nevertheless, insufficient in the light of the new theory of ionization.

The first one implies an indicator and since not all of the many indicators respond to the same degree of acidity or alkalinity, the impracticability of such a definition is obvious. A substance may be acid to litmus and alkali to methyl red, alkali to litmus and acid to phenolphthalein. Furthermore this definition does not take care of the transitional value, i.e., of the value for instance when the color changes from a distinct blue to a distinct red in case of litmus and vice versa.

Disregarding the taste property for an obvious reason and characterizing the third definition as indefinite because precisely as the acid is defined in terms of a base so is a base defined in terms of an acid, we may pass on to the fourth. This one sounds more scientific and appears to be more correct than the others. It is, however, very far from being true, for the most concentrated aqueous solutions of bases contain some hydrogen ions (Clark, p. 21). This consideration follows from the equation.

$$[H^+] \times [OH^-] = Kw$$

$[H^+]$ = hydroxide ion concentration
$[OH^-]$ = hydroxide ion concentration
Kw = constant for water

In order to satisfy this equation, one of the variables must increase when the other decreases but neither one in an aqueous solution can ever be reduced to zero because Kw is constant and greater than zero. The mere assertion then that a solution containing hydrogen or hydroxide ion is an acid or a base, respectively, is not sufficient for the identity of the same. If to this quality factor a quantity factor be added, the resulting definition will be satisfactory. In other words, we must arbitrarily assume some definite amount of hydrogen ion for the neutrality point; an increase in this value will cause acidity; a decrease, alkalinity. Such an arbitrary number is suggested by the above equation. The constant Kw was found to be approximately 1 : 100,000,000,000,000 which means that in a liter of pure distilled water the product of [H+] by [OH⁻] is always approximately equal to $1:10^{14}$. Since in the case of the dissociation of water for every hydrogen ion there must be a hydroxide ion and the factors on the left side of the equation are equal, the number on the right side of it is to be resolved into two equal factors also. $1:100,000,000,000,000 = 1:10,000,000 \times 1:10,000,000$ *or* $1:10^{14} = 1:10^7 \times 1:10^7$. It is hereby proposed then that the quantity $1:10^7$ may be introduced into the definition of an acid: An acid is a substance which when dissolved in water yields a greater concentration of hydrogen ion than $1:10^7$ gms. per liter; a base is a substance which when dissolved in water yields a smaller concentration of hydrogen ion than $1:10^7$ gms. per liter. In other words the difference between an acid and a base is not of kind but of degree, depending not on the mere presence of hydrogen ion but on a definite amount of it.

The definition was dwelt on at length because it is the basis for a better appreciation of the importance of H-ion determination and for a clearer understanding of the meaning of a pH. This shall presently be discussed briefly.

A normal acid is one which contains in a liter of solution one gram of ionizable hydrogen. It makes no difference whether the acid is monobasic like HCl, dibasic like H_2SO_4, or tribasic like H_3PO_4, a normal solution of it should contain one gram of hydrogen in one liter of solution.

A. $\frac{N}{1}$ (Normal) 1 gm. H per liter $\frac{1}{10^0}$

B. $\frac{N}{10}$ (tenth normal) $\frac{1}{10}$ gm. H per liter $\frac{1}{10^1}$

C. $\frac{N}{100}$ $\frac{1}{100}$ gm. H per liter $\frac{1}{10^2}$

D. $\frac{N}{10,000,000}$ $\frac{1}{10,000,000}$ gm. H per liter $\frac{1}{10^7}$

E. $\frac{N}{100,000,000,000,000}$ $\frac{1}{100,000,000,000,000}$ gm. H. per liter $\frac{1}{10^{14}}$

For convenience we use the exponents of the denominators in combination with the symbol pH (potential hydrogen) to denote the corresponding normalities, that is, the pH value is equal to log $\frac{1}{[H+]}$.

Thus the normality in line A is denoted by pH0
" " " B " " " pH1
" " " C " " " pH2
" " " D " " " pH7
" " " E " " " pH14, etc.

In accordance with our definition of an acid pH7 denotes a neutral solution, pHO, a normal acid and pH14, a normal alkali.

The pH value of a solution is determined electrometrically and colorimetrically. In the following pages only the colorimetric methods will be dealt with, not in all their details, but only in so far as to point out some discrepancies resulting from the recommendations of the several authors.

Comparison of Colorimetric Methods

There are three principal systems which are being used at present in the colorimetric method for the testing of the concentration of hydrogen ion in culture media. First, the Walpole system, which comprises the range of pH 2.7—6.5 and which consists of a mixture of standard solutions of acetic acid and sodium acetate. Second, the Sorensen standard phosphate solutions system which covers the pH range from 5.8 to 8.4. This range is the most useful as far as culture media are concerned since it is within this range that most of culture growth is possible. The standards are prepared by mixing definite amounts of dibasic sodium phosphate and monobasic potassium phosphate solutions. Third, the Medalia system, which even though not thoroughly scientific as the other two, is nevertheless of great importance on account of the large range of pH of which it is taking care (1.2—9.6) and on account of the comparatively little difficulty encountered in the preparation of the standard solutions. These are made up of hydrochloric acid and sodium hydroxide of known percentage or normality.

It is obvious that if any systematic work and accurate results are to be obtained, the pHs of the three systems especially at the overlapping points, should coincide. Not only is this coincidence necessary when a medium is tested before and after autoclaving, where the pH value falls or rises to such a point that two systems must be used for the two tests, but it is also of great importance when a comparison of tests or standardizations, done in different laboratories, is to be made.

In undertaking this work it was sought to establish first, where variations or deviations occurred and then if possible to so alter the standards as to make the systems agree. One of the systems, the phosphate solutions, was standardized against a potentiometer and these solutions in turn were used as standards for comparsion with the other two systems.

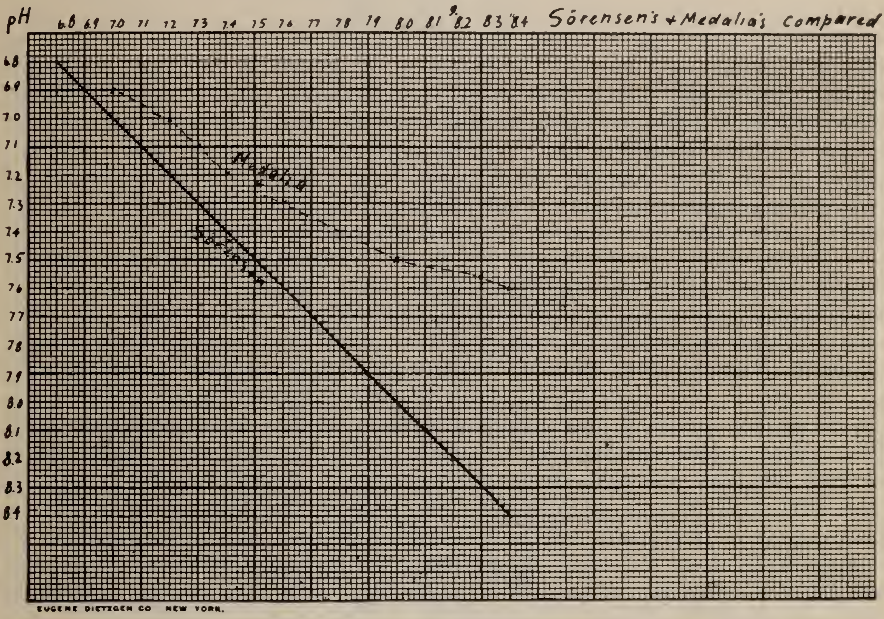
As regards the indicator used for the estimation of the pH, it should be pointed out here that there is a disagreement among the authors as to the percentage of the phenol red in solution and as to the solvent to be used in making up the indicator solutions. Medalia recommends 0.04% solution of phenol red for the phosphate standards; Park & Williams recommend 0.02% solution of phenol red. In this laboratory a 0.04% solution for both the Medalia and Sorensen systems has been in use.

A set of standard tubes of the range pH (6.8—8.4) were made up in each of these two systems. Different media of various pH values were tested against both systems under the same conditions of temperature, quantity, concentration of indicator, age of standards, etc. About one hundred tests were made but only sixty recorded. Only the averages of the reading are given below.

TABLE 1.

Medalia pH	Sörensen pH
6.9	6.8
6.9	6.9
6.9—	7.0
6.95	7.1
7.0	7.2
7.1	7.3
7.2	7.4
7.2	7.5
7.3	7.6
7.35	7.7
7.4	7.8
7.4	7.9
7.5	8.0
7.5—	8.1
7.5—	8.2
7.55	8.3
7.6	8.4

From an examination of this table it can be shown that not only do the values differ widely but the difference increases directly as the pH values. In the accompanying graph the values of the two systems are plotted against each other. The straight line represents the normal comparative values while the broken line shows at a glance the abnormal conditions. It was observed, however, that the end of the broken curve is midway between the ends of the horizontal line and the normal curve.



GRAPH 1—Showing comparative values by Sorensen's and Medalia's systems.

Now, since in the Medalia system only the amounts of indicator are varied while the solutions are of the same strength and amount in each tube, why should it not be possible to halve the intensity of color and make it conform to that of the Sörensen system by simply halving the quantities of the phenol red indicator solution? The affirmative answer to this question was verified by experiment. In several preparations made in this manner the expected results have become true. The equivalence of the intensities of the colors in both systems was proved beyond question.

The restoration of Medalia's standards was the result of this investigation. It resulted also in a great improvement upon this system which proves to be economical, saving time and labor and more reliable as far as accuracy is concerned. A glance at the following tables will aid in seeing the improvement.

TABLE 2

As recommended by Medalia (0.04%) phenol red solution.

	6.8	7.0	7.2	7.4	7.5	7.8	8.0	8.2	8.4	
Amounts of 0.04% phenol red alcoholic solution in each tube ...... cc.	0	.05	.1	.15	.2	.25	.3	.35	.4	Each tube contains 10 cc of $\frac{N}{100}$ Na OH
	.4	.35	.3	.25	.2	.15	.1	.05	.0	Each tube contains 10 cc of 0.1% HCl*
SUM	.4	.4	.4	.4	.4	.4	.4	.4	.4	

*This is made up by simply mixing 1 c. c. of concentrated HCl with 999 c.c. of water. It is not a true % solution. It is about .037%.

TABLE 3.

MODIFIED AND IMPROVED 0.04% SOLUTION

	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0	10.2	
Amounts of 0.04 % phenol red alcoholic solution in each tube	0	.025	.05	.075	.1	.125	.15	.175	.2	.225	.25	.275	.3	.325	.35	.375	.4		Each tube contains 10 cc. of $\frac{N}{100}$ Na OH
	.4	.375	.35	.325	.3	.275	.25	.225	.2	.175	.15	.125	.1	.075	.05	.025	0		Eachtube contains 100 cc. of 0.1 % HCl
SUM	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4		

Notice, in examining tables 1 and 2 that the pH range was doubled for the phenol red indicator. Instead of the three indicators previously used for the range 6.8—9.6 which use was a cause of some of the discrepancies and irregularities, we are now enabled to use only one indicator with a uniform gradation and for a more extended range. This was made possible by halving the differences of amounts of indicator added to two successive alkali tubes.

It should be noted here that a little percussion, a little warming and shaking will help in dissolving the phenol red powder. Also, only small amounts of this should be made up at a time because it gradually loses its coloring power. New standard tubes with two weeks old indicator were found to have less color than the 2 weeks old standard tubes with the same indicator. The change must have occurred in the indicator solution not in the standard tubes. Another very important precaution is to keep the stoppers of the standard tubes absolutely dry. A stopper moistened by the solution in the tube will cause a change in color in a very short time. This has been here amply verified.

Conclusion:—Medalia's system cannot be used without a radical change in it. As recommended by Medalia without improvement the system must be condemned. Clark in his "The Determination of Hydrogen Ions," p. 131, calls Medalia's method misconceptions of theory and practice. Gillespe says: "The proposed tables (Medalia's) are incorrect. . . . the plan on which they are based lacks a solid foundation and it is not supported by enough data." Here they were proven to be in complete disagreement with the other reliable systems. However, it is advantageous and practical to use the standards of Medalia *with the modifications as here recommended;* (1) on account of the fact that for some reason the phenol red standard tubes keep their color for a very much longer period in the case of hydrochloric acid and sodium hydroxide than in the case of phosphates; (2) because of the extended range of pH in this system; and (3) because of the possibility of using a single indicator instead of three or four. Consequently the attempt was made to adjust it by varying the amounts of the indicator from what had been recommended by Medalia. The standard modified tubes are now in complete agreement with the other standards, at least between the values 6.8—8.4 and probably correct up to 9.6.

THE ELIMINATION OF THE PRECIPITATE FROM PHOSPHATE BROTH MEDIUM.

Jacob Weinberg

When phosphate broth medium is prepared according to the formula devised by our laboratory following the general directions of Dochez, Avery and Lancefield a precipitate invariably appears in the medium after autoclaving regardless of the elaborate filtering and regardless of the fact that after such a filtering but before sterilization the medium appears to be very clear.

The precipitate thus appearing has been reported to us by several visiting bacteriologists as well as by our own serologic workers as a great nuisance in antigen work where this medium is mostly used.

A very slight and simple modification has given excellent results in about forty preparations. Where the results were not perfect some deviation from this modification was found to be the cause. There are three points to be observed in this modification. First the bulk of the medium before it is filtered should be autoclaved; second such autoclaving should be at a minimum pressure of 20 lbs. for 30 minutes; and third the final sterilization should be under a *MAXIMUM* pressure of 15 lbs.

For the benefit of those who desire to have the details of the preparation of the phosphate broth a complete formula is here introduced.

Infuse chopped lean beef, which has been freed from fat and tendon, in the ice-box over night. One pound of beef in a liter of water will yield a good infusion. This is boiled until coagulation takes place. A few minutes is sufficient. Strain over cheese cloth. Add peptone (Fairchild's) to make one per cent. solution. Add sodium phosphate to make a 0.2% solution. Heat until all the peptone is dissolved. Adjust the reaction to pH 8.2; 33 c.c. of normal sodium hydroxide per liter of medium is the usual amount required for this adjustment. Heat the entire bulk of the medium in the autoclave under a pressure of 20 lbs. for 30 minutes. This rather high pressure is necessary to throw out at this stage all the precipitable material. If this is not followed precipitation will take place in the final sterilization and cause the endless trouble and interference with the serologic tests already referred to above.

The medium is now filtered through cotton and paper two or more times, depending upon the kind of paper used, and the reaction again adjusted to pH 7.8 by adding one c.c. of normal medium hydroxide per liter for every 0.1 pH lower than 7.8. It is important that the media shall be hot when filtered. The clear medium is bottled and sterilized for 30 minutes at a pressure of *not higher than 15 lbs.* The medium thus prepared ought to have no sediment or cloudiness whatever. The final pH should be 7.5 but 7.6 or 7.4 is satisfactory.

UNSUCCESSFUL ATTEMPTS TO CURE OR PREVENT TUBERCULOSIS IN GUINEA PIGS WITH DREYER'S DEFATTED ANTIGEN.

Published in The American Review of Tuberculosis, 1926, Vol. XIII, pp. 114-123

Mary Nevin, Florence R. Bittman and Elizabeth Lee Hazen

When Dreyer's work on the therapeutic value of a "defatted" vaccine, made from the *Bacillus tuberculosis,* was published, it created quite an interest in the medical world, which looked forward to a more extensive repetition of the work by numerous investigators and a possible verification of all that Dreyer had claimed.

On account of the fact that Dreyer had used so few animals, and, according to our opinion, insufficient controls, we decided to repeat his experiment, using a greater number of animals and more controls, and at the same time to test the prophylactic as well as the therapeutic value of the vaccine.

Summary of Results

According to our experiments, the defatted vaccine prepared according to Dreyer's method produced no beneficial results in tuberculous guinea pigs when treated either therapeutically or prophylactically. On the contrary, the animals treated with the vaccine succumbed to the infection earlier than the controls that received no vaccine treatment. This seems to indicate that the resistance of the animals is lowered by the antigen treatments. Bronfenbrenner and Straub, and Kettel have also noted this fact in their experiments.

The vaccine treatment was generally followed by suppurative lesions, in only a few instances healing over with the crust formation mentioned by Dryer. In no instance, however, was this formation followed by recovery. All of our animals succumbed to the infection.

For bibliography see the original article.

IMMUNIZATION OF A HORSE AGAINST BOTULINUS TOXIN FROM B. BOTULINUS (NEVIN. TYPE B. AND ORR TYPE A).

Edwin J. Banzhaf and Herman Gerber.

The potency of the different lots of toxin used during the course of immunization varied from 0.001 to 0.00005 of a cc. The horse's resistance to botulinus toxin being unknown, the immunization was started cautiously. The first injection was 1/10 of a guinea pig M.L.D. (subcutaneously) on 3-18-20. The injections were increased every second day 100 per cent. Temperature reactions were slight, usually less than one degree, the horse showed no other disturbances and at all times during the entire course of immunization ate his full allowance of rations. After the 12th injection the increase of the toxin dose was dropped to 50%; after five weeks of immunization a test bleeding was taken and potency found to be about 1 unit per cc. Injections were continued at a 25 per cent. rate of increase. The 5-10-20 six liters of blood were drawn from the horse, potency 5 units. The sixth bleeding on 8-26-20 showed a potency of 200 units per c.c. After the ninth bleeding the horse was very much emaciated. The toxin injections from Nevin type B. were discontinued and an attempt was made to immunize the horse against botulin toxin (type A, Orr). The first injections was one guinea pig M.L.D. and increased 100 percent every second day. Three weeks later the horse was found down in the stall and was bled to death.

On November 29th, 1920, immunization was started in another horse against botulinus toxin (type A, Orr). The method of immunization of the previous horse was followed with the exception that the first injection was one guinea pig M.L.D. instead of 1/10 of an M.L.D. On January 13th, 1921, the horse was bled, and it tested 175 units per c.c. 500,000 M.L.D.s was the last amount injected previous to bleeding.

The fourth bleeding was taken February 28th, 1921 and tested 250 units per c.c. It was found that the botulinus antitoxin could be refined and concentrated by the Banzhaf method.

ON THE CLAIM THAT SOME TYPHOID-PARATYPHOID STRAINS SURVIVE THE MILK PASTEURIZATION.

Published in The Journal of Infectious Diseases, 1921, Vol. XXIX, pp. 310-312

Charles Krumwiede and W. Carey Noble

The statement of Schorer and Rosenau[1] that the thermal death points of the pathogenic organisms that may render milk dangerous have been determined with precision in many laboratories and that an actual exposure to 60° C. for 20 minutes would suffice to kill such nonspore-bearing organisms as B. diphtheriae, B. tuberculosis and B. typhosus voiced the general conclusion of all who had worked on this subject. Although the practical application of these facts to the pasteurization of large volumes of milk was another problem, exposure to this temperature, for the time given, had been uniformly considered as adequate, and this aspect of the subject was looked on as closed.

Recently, however, Twiss[2] has reopened the subject and used bacteria of the typhoid-paratyphoid group as test organisms. Her contention is that, in actuality, the temperatures of pasteurization are inadequate; that the method of using small samples of the heated milk for the determination of the death of the bacterium present does not exclude the possibility of the survival of a few organisms. She asserts that if the whole test sample (100 c.c.) be incubated it will not infrequently be found that a few bacilli have survived exposure to 60° and even to 65° C. for 30 minutes.

The practical importance of the question immediately presented itself, and we began testing a series of cultures from different sources. Twenty-seven typhoid cultures recently isolated from carriers, 7 paratyphoid A cultures, 12 paratyphoid B. culutres and 4 enteritidis cultures were subjected to experiments, using the ordinary temperature of pasteurization.

Milk was sterilized in the autoclave, cooled and tested for sterility. It was then distributed in measured quantities in sterile containers. The containers were placed in wire baskets on a perforated tray in a water bath, and the temperature of the bath and the milk raised to 60° C. After this temperature was reached, suspensions of the cultures in salt solution were poured into the milk. Rubber stoppers were inserted, the bottles shaken vigorously and quickly returned to the water bath in which they were completely submerged. The temperature of 60° C. was held during the pasteurization period of 15 minutes. Rapid cooling was accomplished by plunging the samples into a bucket of cold water held under an open faucet. This method of pasteurization excluded the preliminary heating of the bacteria while the temperature of the milk was being raised. In every one of these tests we used a volume of 100 c.c. of sterilized milk infected with the cultural growth from two 24-hour agar slants suspended in salt solution.

The inoculated milk samples were then incubated for 48 hours at 37° C. to allow multiplication of any organisms which survived. After incubation, samples in duplicate, 0.5 c.c. and 0.1 c.c. were plated in agar and 2 broth tubes were each inoculated with 0.1 c.c. Russell's medium was also inoculated from the infected milk, as well as from the inoculated broth tubes.

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[1] Jour. Med. Research, 1912, 26, p. 127.

[2] Journ. Infect. Dis., 1920, 26, p. 165.

None of the micro-organism tested survived a pasteurization period thus limited closely to 15 minutes at 60° C.

These experiments confirmed our previous experiences, as well as those of others. Whether or not there might be more resistant members of the typhoid-paratyphoid group could not be excluded as a possibility, although this seemed improbable. To exclude the possibility that Twiss was working with unusually heat resistant strains, we obtained some of the strains which in her experiments seemed to survive the usually accepted pasteurization period.

The resistance of these strains was tested in several ways. One set of experiments with 100 c.c. of milk was carried out as described, and no growth was obtained. Then, larger volumes were inoculated very heavily by using for each bottle the growth from 10 agar slants. Whether the milk was inoculated before or after reaching 60° C., the results were the same. No growth was obtained after 20 minutes or even after 10 minutes' heating at 60° C.

Repetitions of these tests, not recorded here, have given the same negative results.

It would seem that the apparent heat resistance of the strains reported by Twiss was due to the test method employed, namely, the use of cotton plugged flasks submerged to twice the depth of the milk. The possibilities of discordant results from this open method have been pointed out by Smith[3] and others.

CONCLUSIONS

There is no evidence that bacilli of the typhoid-paratyphoid group, even in small numbers, will survive heating to 60° C. for 20 minutes. To insure actual heating of all the sample and to exclude other sources of error in laboratory pasteurization tests, the sample container should be completely submerged in the bath.

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[3] Jour. Exper. Med., 1899, 4, p. 233.

A STUDY OF THE PRACTICAL VALUE OF THE FROST LITTLE PLATE METHOD IN THE ROUTINE COLONY COUNT OF MILK SAMPLES

Published in The American Journal of Public Health, 1922, Vol. XII, pp. 478-487

Hazel M. Hatfield and William H. Park

The method of growing bacteria in solid media on sterile slides was applied by W. D. Frost in 1915 to the routine bacteriological examination of milk. It was called by him the "little plate" method.

This method is a modification of the standard plate method for milk analysis in that the standard agar for milk work is used and the result obtained is a computation of the number of colonies per cubic centimeter of milk developing under conditions essentially those of the standard plate procedure. Since 1915, Frost, working at the University of Wisconsin, has repeatedly modified and simplified the technic and improved the necessary apparatus.

The present technic of the little plate method is not difficult. An assistant with good general laboratory training can learn to make the test without difficulty; workers with less general experience gain proficiency more slowly, but a few days of practice will usually suffice for accurate results.

The method[1] in brief is described by its author as follows:

Two areas of four square centimeters each are marked off with a wax pencil on an ordinary microscopic slide which has been carefully polished. The slide is sterilized by passing it back and forth through a Bunsen flame. It is then laid on the flat top of a copper box containing water at a temperature of 42° to 45° C. The milk sample is shaken gently to avoid the formation of foam, then 0.05 (1/20) c.c. of the milk is measured from a "serum pipette" (a narrow, accurately calibrated pipette measuring 0.2 c.c. graduated to 0.01 cc.) onto the ruled area at the left of the slide, and the same amount on the right end of the slide. An approximately equal amount of sterile nutrient liquified agar at 42° to 45° C. is added by allowing one drop to fall from a 1.0 c.c. pipette upon each measured drop of milk sample.

The milk and agar are thoroughly mixed with a sterile loop and carefully spread over the marked-off areas. This gives duplicate films. The preparation is set aside to solidify on a level surface under cover, and then, before the agar begins to dry down, the slide is placed in a moist sterile chamber. This moist cabinet is set in the incubator. The period of incubation should be long enough to allow the bacteria to grow into definite colonies, although they need not necessarily be visible to the naked eye. It is stated that four hours incubation at 37.5° C. allows the formation of good-sized colonies if the bacteria are actively growing in the milk at the time of testing. If the milk has come from cold storage, has been pasteurized, or is fresh and contains few bacteria, then seven or eight hours are necessary. If there is no particular hurry about the results, the plates may be left in the incubator from one day to the next, i.e., for fifteen or sixteen hours.

When the slides are removed from the moist chamber after incubation, they are dried immediately at a temperature of about 95° C. If only a few slides are to be dried, they may be laid on the surface of the above mentioned copper box, which will give a temperature of about 95° C. if the box is filled with boiling water. Where large numbers of slides are to be dried, an electric oven is necessary. This temperature dries the agar films quickly enough to prevent the film from cracking or peeling. The drying is accomplished in five minutes or less.

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[1] Frost, W. D., Improved Technic for the Micro or Little Plate Method of Counting Bacteria in Milk. Jour. Inf. Dis., XXVIII, 2, p. 176.

The slides are now ready for staining. They are first immersed for a minute or two in 95 per cent. alcohol containing 10 per cent. of glacial acetic acid to prevent the agar background from taking a deep stain. The acid alcohol is drained off and the slides are then put into the stain. This may be either Loeffler's methlylene blue diluted with three times its volume of distilled water, or thionine blue made up with carbolic acid and distilled water with the addition of acetic acid. The stain will act sufficiently in about two minutes, but no harm will result from a longer exposure. The slides should be thoroughly washed in tap water, then dried at about 95° C. on a hot plate or in an electric oven as described above.

The counting is done under a compound microscope. If the colonies are small or very numerous the higher powers must be used; if the colonies are larger and fewer the low power may be used. A number of fields are counted and an average count per field obtained. In order to express the result of the count in terms of "colonies per c.c." of milk, it is necessary to know the area of the microscopic field. To determine this, the diameter of the field is read off on a stage micrometer, the radius is obtained by dividing the diameter by two and the following geometrical formula applied:

The area of a circle=radius squared × 3.14159. Then divide the area of the microscopic field into the total area of the film (4 square centimeters) and multiply the result by 20 because only 0.05 c.c. of milk is used to make the test. The result is a constant by which the average count per field must be multiplied in order to convert it into "colonies per c.c." of milk. This multiplier is a constant for each combination of lenses so tested but must be determined again for each microscope and for each new combination of lenses.

Heavily contaminated milk samples require dilution before this test can be carried out. The dilution advised is 1.0 c.c. of the milk to be tested in 9.0 c.c. of sterile milk. After thorough mixing, the diluted sample is used in exactly the same way as the ordinary sample. Another way, simpler than this, is also recommended: 0.01 c.c. or 0.02 c.c. of the undiluted milk sample is pipetted onto the ruled slide, a drop of warm sterile milk and the usual drop of agar added,—the whole mixed and spread in the usual way.

The advantages claimed by Frost for the little plate method are:

1. The time required to set up the little plate test is less than that needed for the standard plate test.

2. The incubation period is shortened so that routine results may be obtained in twenty-four hours and, in special cases, results may be obtained the same day.

3. The technic of the little plate method is simpler than the technic of the standard plate method.

4. The little plate method requires less material in the way of glassware and media. There is a corresponding saving of labor and expense in preparing these supplies.

5. It permits a study of a relatively large amount of milk, usually 0.05 c.c.

6. The slides are permanent preparations and can be filed for record and re-examined whenever the need arises.
7. The method furnishes direct information about the types of organisms present, their relative numbers, etc.
8. It is a method easily adapted to field work.

To determine how far the Frost little plate method is applicable to the work of a large routine milk laboratory run for administrative purposes, we have carried out an extensive series of tests on all grades of milk, controlling the test in all cases by standard plate counts. Whenever it was possible to do so, the standard plate was made in duplicate. A few minor changes have been made in the Frost technic where such modifications would expedite the work or make the test more like the standard plate method.

Our most important modification of Frost's technic concerns the shaking of the milk sample. Frost tips the sample bottle from end to end slowly enough to avoid the formation of foam, since air bubbles drawn into the pipette would interfere with the accuracy of measurement. For routine work, the manner of shaking milk samples is minutely prescribed by standard methods; it is a vigorous shake and will break up clumps more than the shaking recommended by Frost. In order to make a comparative study, with the standard plate results as the basis for comparison, it seemed essential to shake the milk sample in the same manner for both tests. In our laboratory, therefore, the sample is shaken in the manner prescribed by standard methods and then both tests are immediately carried out. Tipping the bottle after shaking causes the foam to float to one side and the milk may be drawn into the pipette without including air bubbles, thus overcoming the objection mentioned by Frost.

To save the time consumed in ruling the slides, a Wolf-Hügel counting plate is placed upon the warm box and the sterile slides placed upon the counting plate. The ruled lines of the counting plate are clearly seen through the slide and the milk-agar mixture is carefully spread over four square centimeters of the slide as defined by the lines beneath. Although we lose the advantage of having the mixture flow back from a greased line, we accomplish a slight saving in time on each test and this saving becomes considerable as the number of tests increases.

After staining the film, we prefer to decolorize slightly. This technical point was mentioned by Frost in his earlier articles, but is not mentioned in his most recent article. The count seems to be uninfluenced by decolorization if this is not carried too far. A few seconds in alcohol are all that should be allowed. Films that have been overdecolorized may easily give too low a count, as unstained colonies cannot always be picked out with accuracy.

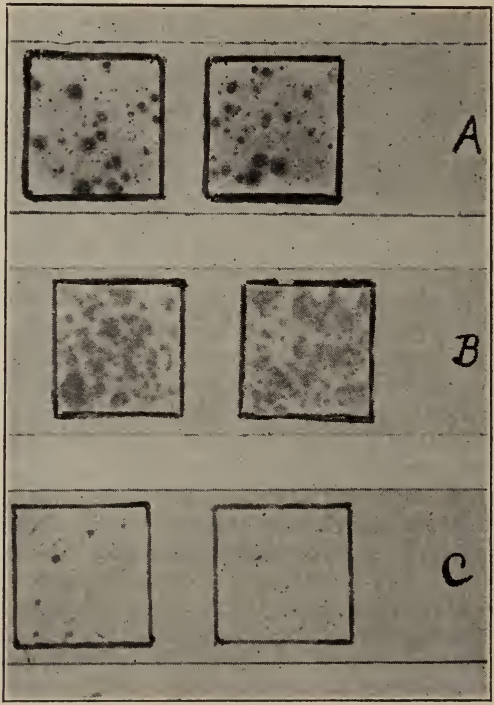
The accompanying photograph shows the stained preparation ready for counting.

In all the work used as the basis for this article, only one time-period for incubation has been employed. The moist chamber containing the slide preparations was put into the incubator at 5 p. m. and was taken out at about 8 a. m., thus making a fifteen to sixteen hours incubation. As pointed out by Frost, it is desirable to incubate over night when dealing with the test in a routine way, otherwise it is necessary to be at the laboratory out of hours. With a large volume of routine work, the overnight incubation is important to the indorsement of the method. Although Frost's work is based upon an incubation period of four to eight hours, the overnight incubation is equally recommended by him. It is probable that any difference in results is rather in the size of the colony than in the count.

When we began this work, colony counts were made in an absolutely mechanical way by counting five or ten fields of each film, going diagonally across the film in both directions and averaging these results. As the work went on, however, and our experience grew, we found that with ordinary care in making the films, the colonies were so evenly distributed that equally accurate results could be obtained by counting two or three representative fields from each film. It is preferable to do this with a mechanical stage.

PLATE SHEET

The appearance of the little plates after staining is shown in the accompanying photograph.



Little plates in duplicate on slides after staining (fifteen hours incubation). *A*—Moderate number of colonies; *B*—High count, more uniform colonies; *C*—Small number of colonies. (Reprinted by courtesy of The American Journal of Public Health.)

Some of the assistants in our laboratory have also been tried out on the counting. They have required little practice before obtaining counts very like our own. It must be borne in mind, however, that these girls have had years of experience in counting milk plates. Their opinion is that the stained little plate preparation is easier to count and entails less eye-strain than does the standard milk plate.

As a rule, the count is not radically affected by the choice of an objective. (See Table 1.) Of course, where the count is very low, the oil immersion is impracticable because many fields will show no colonies, hence it is difficult to get a true average count per field, and the factor for multiplication is so large that a slight variation in the average count is multiplied enormously.

TABLE 1.
COMPARATIVE COUNTS ON MILK SAMPLES WITH SPECIAL REFERENCE TO THE USE OF VARIOUS OBJECTIVES.

	COLONIES PER C.C. OF MILK					
	Frost Little Plate Method				Standard Plate Method	
Sample No.	Amt. Tested	No. 3 Objective	No. 6 Objective	Oil Immersion	Dilution Used	Count per c.c.
1	.05 c.c. .01 c.c.	Overcrowded 2,741,892	 2,624,958	2,360,000 2,000,000	.... 1 1,000	 2,500,000
2	.05 c.c. .01 c.c.	37,996 *	60,416 *	90,000 *	1:1,000	65,000
3	.05 c.c. .01 c.c.	172,983 114,433	183,330 187,497	220,000 too few	1:1,000 1:10,000	224,000 210,000
4	.05 c.c. .01 c.c.	34,330 *	70,832 *	80,000 *	1:1,000	45,000
5	.05 c.c. .01 c.c.	Overcrowded 4,452,000+	1,894,279 4,977,989?	1,973,552 3,788,515	1:10,000	11,000,000
6	.05 c.c. .01 c.c.	595,720 1,297,440	572,689 678,416	722,461 704,840	1:10,000	1,200,000
7	.05 c.c. .01 c.c.	Many millions Many millions	Many millions Many millions	Many millions Many millions	1:100,000	223,200,000
8	.05 c.c. .01 c.c.	Overcrowded 11,872,000	4,390,000? 14,096,960?	Col. run together 7,224,610	1:100,000	27,000,000
9	.05 c.c. .01 c.c.	1,225,360 1,250,800	1,057,272 1,718,067	1,074,881 1,057,250	1:10,000	2,400,000
10	.05 c.c. .01 c.c.	Many millions Many millions	Many millions 10,572,720	Col. run together 9,162,920	1:10,000	124,620,000
11	.05 c.c. .01 c.c.	Overcrowded 3,697 280	1,392,075 2,731,286	1,797,342 2,746,655	1:1,000	3,200,000
12	.05 c.c. .01 c.c.	529,900 712,320	405,288 616,742	334,799 792,945	1:1,000	776,000
13	.05 c.c. .01 c.c.	Many millions Many millions	Many millions Many millions	Many millions 32,775,060+	1:10,000	137,640,000
14	.05 c.c. .01 c.c.	3,595;520+ 6,784,000+	4,365,330? 5,286,360?	2,625 529 Colonies indistinct	1:10,000	10,800,000
15	.05 c.c. .01 c.c.	850,120 1,036,680	740,090 660,795	599,114 Growth too diffuse	1:10,000	1,100,000
16	.05 c.c. .01 c.c.	295,104? 560,740	208,376 *186,050	881,050? Diffuse growth *176,210?	1:100 1:1,000 1:10,000	273,000 400,000 350,000
17	.05 c.c. .01 c.c.	62,208 58,300	54,573 *	* *	1:100 1:1,000 1:10,000	36,000 51,000 120,000
18	.05 c.c. .01 c.c.	24,168 27,560	24,807 *	* *	1:100 1:1,000 1:10,000	24,000 28,000 100,000
19	.05 c.c. .01 c.c.	366,316 312,640	282,796 *	328,925 *	1:100 1:1,000 1:10,000	227,500 584,000 500,000
20	.05 c.c. .01 c.c.	35,616 46,640	* *	* *	1:100 1:1,000 1:10,000	28,800 53,000 60,000

Samples Nos. 3, 5, 7, 10, 13, 16, 19 were diluted 1:10 to makes samples Nos. 4, 6, 8, 11, 14, 17, 20, and these in turn were diluted 1:10 to make samples Nos. 9, 12, 15 and 18.

NOTE: These milks contained an excessive number of lactic acid bacteria, having been incubated at room temperature over night.

*Too few for accurate count.

If the milk has a very high bacteria content, the field under the low power is too overcrowded to be accurately counted and the oil immersion is much more satisfactory. In the latter case the count may be too low even with the oil immersion because of the inhibition of colony development due to overcrowding, hence the testing of a smaller amount of milk is indicated.

In either case, the important fact for grading purposes is easily ascertained—a very high or a very low count is quickly recognized and a medium count can be made with any objective. Occasionally a milk is found which gives a higher count when the magnification is increased. This may be due to a different type of milk or to artifacts. (See Table 2.) These cases require further study. In this connection, it must be borne in mind that the oil immersion shows the individual bacteria and that clumps of these cannot, at times, be differentiated from beginning colonies. The possible effect of an unusual flora is shown by the following case:

Thirty-nine samples of pasteurized milk, all from one source, were run in parallel by the two methods. The standard plates gave counts ranging from 62,000 to 710,000 per c.c. and showed apparently an almost pure culture of an organism producing extremely small colonies. The Frost plates all gave counts under 10,000 per c.c. when examined with the No. 3 objective. With the No. 6 objective the counts were somewhat higher. With the oil immersion lens very few colonies could be found, but many fields showed a mass of organisms numbering many hundreds, all of the same general appearance, but no denser or more deeply stained in one part of the mass than in another.

Table 2 gives results on five of these samples picked at random.

TABLE 2.

EFFECT OF PRESENCE OF UNUSUAL FLORA

Little Plate Counts Increased with Increased Magnification. Standard Plate Counts Still Higher

		Frost Little Plate Count		
Sample No.	Standard Plate Count	No. 3 Objective	No. 6 Objective	Oil Immersion Objective
894	91,000	6,500	33,333	Diffuse growth instead of colony formation. No Colonies seen.
4062	92,000	6,000	37,499	
7042	62,000	5,333	41,666	
7456	110,000	10,322*	41,666	
599	192,000	2,333	41.666**	

*Possibly much higher count.
**Very indistinct.

In general, the lowest possible magnification consistent with accuracy should be used, because it requires the smallest factors for multiplication and because the oil immersion introduces the danger of including the above-mentioned clumps in the colony count.

The making of proper dilutions in a rapid manner is a matter of great importance. It is true equally for the standard plate and for the little plate that the amount of milk tested should depend upon the probable bacterial content of each sample as indicated by the grade designated on the label. Where the counts are actually far in excess of this, conditions are not suitable for the development of all potential colonies and the count per c.c. is less than it would have been from a higher dilution.

For the better grades of milk, the samples were tested undiluted, using

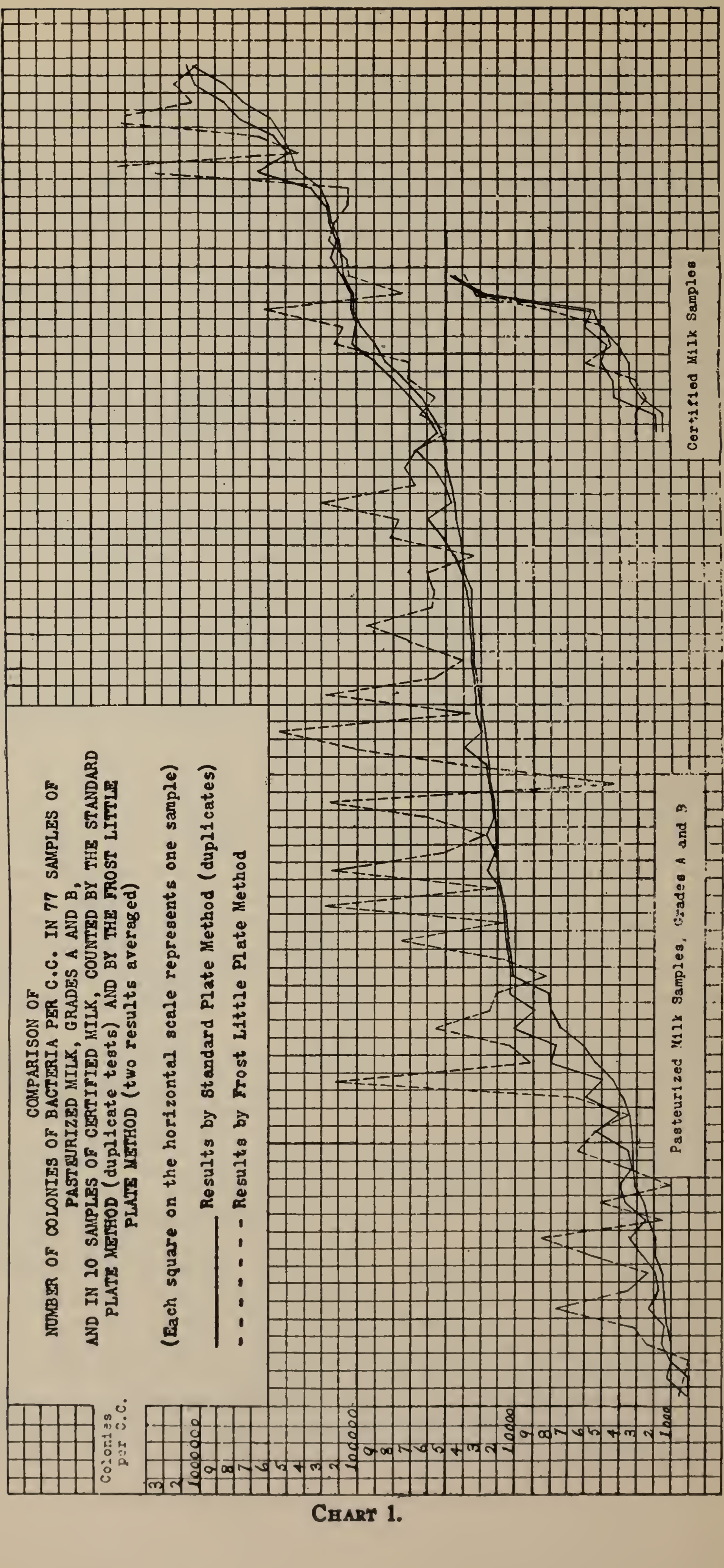
0.05 c.c. of milk and about an equal amount of agar. For the more highly contaminated milks various ways of examining a smaller portion of milk were tried, in order to give the potential colonies room to develop. This, of course, meant a higher factor to be used as a multiplier for the average field. At first, the samples were diluted 1:100, using sterile milk as a diluent; of this dilution, 0.05 c.c. was plated. This technic was unsatisfactory because there were too few colonies in the film.

Next, the milk sample was similarly diluted 1:20 with sterile milk and the usual 0.05 c.c. plated. This method was more satisfactory than the dilution of 1:100, yet often even the 1:20 dilution gave films showing too few colonies. In any case, the making of a dilution was time-consuming and we wished to do the work more directly if possible. To this end we tried the examination of 0.01 c.c. of undiluted milk, adding to it 0.04 c.c. of sterile milk and the usual drop of agar. This procedure was time consuming also and the 0.01 c.c. of milk begins to dry down before the sterile milk can be accurately measured and added.

The method eventually adopted by us for the testing of highly seeded milk samples consists in the examination of two different quantities of the undiluted milk sample, 0.05 c.c. on the left end of the slide and 0.01 c.c. on the right end of the slide without the addition of sterile milk. This has the advantage of simplified technic and the elimination of a sterile diluent with its attendant work of preparation and possibility of contamination. The lessened milk content of one of the finished preparations does not seem to make as much difference in the count as we anticipated. Although it is easily conceivable that milks containing large numbers of certain types of lactic acid bacteria would fail to show a corresponding colony count in the film containing less milk, yet the same objection is true of the standard plate which actually contains even less milk. As this is a study based on comparisons with the standard plate results, we believe that the above procedure is admissible. Moreover, the finding of a reasonable colony count ratio between two films containing different amounts of the same milk sample is reassuring. It is comparable to the colony count ratio between two or more standard plates containing different quantities of a given milk sample. In each case, due allowance is made for inhibition due to overcrowding. For field work, the elimination of a sterile diluent also means fewer supplies to carry about, a less complicated procedure in an improvised workroom and a shorter time required for setting up the test.

The results of our work, on the whole, have been more clear-cut than we had anticipated. The certified milks showed the smallest average variation from the standard plate result. The pasteurized milks and raw milks to be pasteurized gave greater variations, these being divided about equally between results greater than standard plate counts and results less than standard plate count and ranging from —28.6 per cent. to +18.7 per cent. Table 3 presents an analysis of the counts obtained from 241 samples of milk tested by both methods. Only later results are tabulated here because they represent a more experienced handling of the little plate method and the use of 0.05 c.c. and 0.01 c.c. of undiluted samples of raw milk.

These percentages are somewhat higher than those obtained from duplicate standard plates made during the course of this study. The duplicate standard plates gave an average variation of 9 per cent. on 87 samples of certified and pasteurized milk. These duplicate standard plate counts with the corresponding Frost little plate counts are graphcially shown in the accompanying Chart 1.



(Reprinted by courtesy of The American Journal of Public Health.)

Chart 1.

TABLE 3.

CORRESPONDING AVERAGE COUNTS BY THE STANDARD PLATE METHOD AND THE FROST "LITTLE PLATE" METHOD.

Grade of Milk	Legal Maximum Colony Count per c.c.	Number of Samples Included	Average Colony Count per c.c.		Little Plate Variation from Standard Plate
			Standard Plate	Little Plate	
Certified	10,000	22	4,484	4,330	-3.5%
		(24)*	(14,660)*	(16,468)*	(12.4%)*
A. Pasteurized.........	30,000	15	15,220	18.067	18.7%
B. Pasteurized.........	100,000	47	83,874	98,757	17.7%
A. Raw...............	200,000	66	73,403	84,921	15.7%
**B. Raw (country)...	300,000	48	1,377,917	984,182	**-28.6%
B. Raw (city)	1,500,000	41	693,793	591,101	-14.8%

*These entries represent the same 22 samples of certified milk shown above plus 2 very high count samples (see Table 2). It can be seen how quickly the average variation by the two methods is altered when excessively high count samples are included.

**More than half of these samples were 48 hours old and the counts on many were too high for exact findings with the present way of making dilutions for the little plate test.

In Table 4 comparative results by the two methods are given in detail for each grade of milk. These results have been selected from many hundreds of similar results in such way as to convey a fair idea of the usual agreement by the two methods and the degree of occasional disagreement.

It will be seen (Table 4) how rarely the comparative counts fail to coincide in rating the milk above or below standard. In not more than 2.0 per cent. of all samples tested would the legal rating have been altered by the method of counting; the milks were either above or below standard by both methods. The exceptions noted were about evenly divided between those cases where only the standard plate gave a count in excess of grade and the cases where only the Frost little plate gave a count in excess of grade.

Summary.—In the hands of properly trained workers the Frost little plate count is probably as accurate and reliable as the standard plate count for routine laboratory work, especially in the case of pasteurized, certified and Grade A raw milks with the rare exception of milks containing large numbers of certain unusual types of bacteria. Where the counts exceed a million, as they often do for Grade B raw milk (the legal limit for which is 1,500,000 per c.c.), a greater discrepancy between the two methods sometimes appears. Even here the rating is seldom affected by the method used, but it is evident that the dilution technic for the Frost little plates must be further modified to take care of very highly contaminated milks and those containing unusual types of bacteria.

TABLE 4.

Grade of Milk	Legal Maximum per c.c.	Colonies per c.c.	
		Standard Plate	Frost Little Plate
Certified	10,000	5,650	8,100
		4,400	4,300
		3,500	2,600
		4,500	3,100
		5,000	5,900
		5,500	4,800
		1,800	3,100
		1,800	3,100
		*141,200	*147,300
		*112,000	*152,600
		1,600	2,000
		1,800	3,000
		900	300
		1,500	1,600
		*12,000	8,800
		*16,500	*18,900
		3,000	2,500
		1,100	4,000
		3,500	2,000
		3,500	1,300
		6,700	7,100
		10,000	5,600
		2,200	1,100
		2,200	1,000
A. Pasteurized	30,000	*Plate over-crowded	*2,446,400
		4,500	18,900
		12,000	10,900
		9,000	13,900
		3,500	4,800
		2,000	3,300
		*80,000	*93,300
		20,000	19,400
		*38,000	*37,000
		5,200	9,000
		5,000	5,000
		5,000	5,000
		*61,700	*76,600
		13,500	28,900
		3,300	3,000
		7,500	5,000
		8,000	5,000
		2,300	4,000
		*400,000	*144,000
		5,300	6,000
		18,500	24,200
		30,000	*37,900
B. Pasteurized	100,000	28,000	26,100
		43,000	85,800
		3,900	7,000
B. Pasteurized (Continued)	100,000	*147,000	*133,000
		*182,000	*319,900
		*223,000	*273,000
		88,000	50,600
		41,000	33,300
		80,000	100,000
		44,000	37,200
		*170,000	*159,000
		*120,000	86,700
		24,000	24,000
		*640,000	*773,300
		61,500	70,000
		20,000	27,000
		6,600	8,000
		3,900	7,000
		59,000	44,900
		65,000	26,500
		96,000	19,500
		*260,000	*139,200
		*420,000	*599,900
		43,000	64,900
		*110,000	*129,900
A. Raw	200,000	36,000	35,800
		42,000	46,600
		58,000	53,300
		104,000	123,300
		120,000	138,300
		144,000	199,900
		80,500	94,900
		110,000	84,900
		100,000	121,600
		96,000	120,600
B. Raw ..... Collected in Country	300,000	240,000	296,600
		92,000	92,000
		*1,800,000	*1,866,400
		*2,300,000	*2,899,700
		250,000	283,300
		*556,000	*783,300
		*556,000	*599,900
		*500,000	*366,600
B. Raw ..... Collected in City	1,500,000	160,000	186,600
		630,000	491,600
		140,000	199,900
		42,500	49,900
		560,000	473,200
		*1,550,000	*1,606,500
		240,000	233,300
		*2,650,000	*1,733,100
		*2,200,000	*1,666,500
		155,000	166,600
		940,000	1,116,500

NOTE: Stars indicate counts in excess of legal maximum.

Conclusions.—Our present opinion is that both methods are to be depended upon and that, according to the local conditions, one or the other may be utilized.

Whether this test supplants the standard plate method or not, it is a matter of satisfaction to have a second test that can be run in parallel whenever check tests are made.

For bibliography see the original article.

STANDARDS FOR MILK PASTEURIZATION

Published in The American Journal of Hygiene, 1927, Vol. VII, pp. 147-173

By Charles E. North and William H. Park

There has just been reported the occurrence of an outbreak of septic sore throat in Guilford and adjoining towns in Connecticut. During July and August there were 200 cases. Eighty per cent. of those affected were consumers of milk from a single dairy. This milk was raw and unheated. On the farm was a milker convalescing from septic sore throat, through whom some of the cow's udders were infected and from these the milk infection started.

This is only another one of many such instances. Epidemics of typhoid, scarlet fever, septic sore throat, and diphtheria have been so often traced to raw milk that pasteurization of public milk supplies is recognized as a public necessity. The constant menace of bovine tuberculosis adds its weight to this demand. The infections of infants and children by raw milk and the benefits of pasteurized milk for infant feeding are now part of medical knowledge.

But the term "pasteurization" itself still has an indefinite meaning. The temperatures and times originally used by Pasteur for wines and beers are not sufficient for milk. The use of the process for milk, first for the protection of calves and pigs, later on for the protection of infants and children, and finally for the protection of the general public, has resulted in the adoption of different legal requirements by many cities and states. A survey of the legal standards, however, reveals a marked disagreement both as to the temperature of heating and the time of exposure. Many local health authorities have adopted local requirements without an accepted principle as a basis, and without recognizing the definitions used elsewhere. There are at present no uniform standards accepted by health authorities in the United States. As a consequence public confidence in the process of pasteurization and in pasteurized milk has suffered. The lack of such standards is therefore detrimental to public health.

The bovine tubercle bacillus is more resistant to heat than any of the other species of pathogenic bacteria commonly carried by milk. Therefore, standards for pasteurization must be based on standards for the destruction by heat of the bovine tubercle bacillus.

In the classical experiments of Theobald Smith there were 83 samples of milk pasteurized at 140° F. for various periods of time. From these, 83 guinea pigs were inoculated. From the samples heated for shorter periods, the pigs gave positive results. The longer periods gave negative results. Smith's opinion that these data were enough to justify the conclusion that tubercle bacilli are killed at 60° C. (140° F.) in 20 minutes has been universally accepted by bacteriologists.

Russell and Hastings in 1900 tested 30 samples on 60 guinea pigs in one experiment and 18 samples on 36 guinea pigs in another. These data were deemed sufficient to warrant the conclusion by them that tubercle bacilli are killed at 60° C. for 20 minutes.

Rosenau in 1908 tested 87 samples of milk with 87 guinea pigs, and the evidence was sufficient in his opinion to support the conclusion that tubercle bacilli are killed at 60° C. for 20 minutes.

The group of bacteriologists working at Endicott in 1922-23 performed the following tests, which are reported in Bulletin No. 147 of the U. S. Public Health Service:

Temperatures	Number of Samples	Number of Guinea Pigs	Table Nos.
135° F. to 137° F.	27	54	19
130 " 140	27	54	20
134 " 145	122	244	34
141 " 142	19	38	35
130 " 147	90	180	36
142 " 150	64	128	67, 71, 75, 79
138 " 142	92	184	68, 72, 76, 80, 89
138 " 146	106	212	69, 73, 77, 81, 85, 90
138 " 148	171	342	70, 74, 78, 82, 86, 95

These tests show totals of 718 samples tested on 1,436 guinea pigs at from 130° F. to 150° F. This is the volume of data on which Moore, Park, Rosenau, Wadsworth, Armstrong and North base the statement that the tubercle bacillus is killed at 138° F. in 30 minutes, and that 142° F. for 30 minutes is a standard for pasteurization that provides a margin of safety sufficient to protect the public health against milk-borne infections. The data in these tests are more abundant than the data in the tests of Smith, Russell and Hastings, and Rosenau, which no one has yet questioned.

Bulletin No. 147 of the U. S. Public Health Service concludes that the tubercle bacillus is killed at 138° F. for 30 minutes. This is some help in extending the curve below the classical point of 60° C. for 20 minutes. But there still remains some uncertainty regarding the effect of low temperatures on the tubercle bacillus, because no systematic research on the various temperatures below 60° C. has yet been performed.

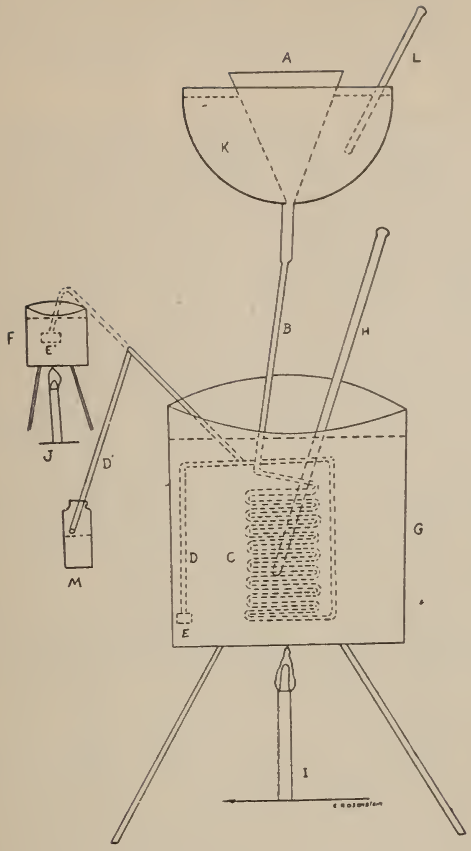
The experiments reported herein were undertaken to supply this deficiency.

It cannot be claimed that all sources of error of previous investigators were eliminated. But plans were made to prevent a repetition of the errors previously enumerated. It is true that standard conditions for the heating and testing of the tubercle bacillus are not yet recognized. Good management and good planning are as important as technical skill in making research work successful. The conditions planned and used in this research were as follows:

Culture. The culture used was of the bovine species known as the "Ravenel" culture, furnished by Dr. M. P. Ravenel to Dr. Wm. H. Park several years ago. This had been kept alive on glycerine agar and was cultivated on glycerine broth until abundant growth was obtained.

Virulence. One one-thousandth of a milligram injected subcutaneously produced in a guinea pig a tubercular abscess at the point of inoculation, also tubercles in the superficial axillary and inguinal nodes, in the lungs, kidneys, and spleen at autopsy, 49 days after inoculation. Also, one one-thousandth of a milligram injected intravenously in a rabbit produced extensive tubercles of the lungs and kidneys and isolated tubercles in the spleen.

Mixture. The growth on the surface of the glycerine broth was ground in a mortar with sterile salt solution until a smooth uniform suspension, so



Heating equipment for testing thermal death points of bovine tubercle bacillus.

A......Funnel supplying tuberculous milk
B......Rubber tubing from funnel to lead coil
C......Lead coil
D......Rubber tubing outlet from lead coil
E......Metal plug at end of outlet
E′.....Metal plug in sterilizing tank
F......Sterilizing tank
G......Water tank for heating lead coil
H......Thermometer
I......Burner
J......Burner
K......Water bath for funnel
L......Thermometer
M......Sample bottle receiving heated milk

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far as possible, was obtained. This was filtered through coarse filter paper to remove any accidental lumps. This suspension was then mixed with sterilized milk, the quantity of tubercle bacilli in each cubic centimeter being about twenty times the quantity already found necessary to kill a guinea pig by the virulence tests.

Equipment. Full consideration was given to all of the varieties of heating equipment used by previous investigators. The primary object of the process was to heat tubercle bacilli uniformly within a fraction of one degree of any chosen temperature for any chosen period of time. The type of apparatus used by MacFadyen and Hewlett in 1897 seemed the most certain to accomplish these purposes. This in principle consists of a coil of small calibre lead pipe, submerged in a hot water bath. At each end of the lead pipe are attached pieces of rubber tubing, one for inlet and the other for outlet. The inlet rubber tube receives the supply of tuberculous milk from a glass funnel kept warm by a warm water jacket. The outlet rubber tube is stoppered with a metal plug which is removable. The lead coil is heated by the water in which it is submerged. The water is heated in a metallic pail or tank to any degree desired, as indicated by a thermometer suspended in the water. There is a small secondary vessel of boiling water to be used for sterilizing the end and metal plug of the outlet pipe. This method of heating was used for all temperatures under 180°. In the three highest temperatures, open tubes over a flame were utilized, and, except for the boiling temperature, are not strictly accurate.

Operation. To operate this heating unit the glass funnel is filled with milk and this is warmed to the desired temperature, and the hot water surrounding it is heated two degrees hotter. The emulsion of tubercle bacilli in milk is heated to about 100° F. The water in the main tank containing the lead coil is previously heated to the desired degree for a length of time sufficient to make certain that the lead coil is thoroughly heated. The rubber tubing at the outlet end, with its metal plug, is submerged under the water and also heated. When the time in minutes and seconds for the beginning of the test is noted and recorded, the milk emulsion of tubercle bacilli is added to the heated milk, the mixture is vigorously stirred for 3 seconds and and allowed to run into the lead coil. To do this the rubber tubing outlet is lifted out of water with forceps, the metal plug removed and held open until the tuberculous milk has flowed by gravity through the lead coil and appears at the outlet. The whole operation takes about 5 seconds. About 10 c.c. of overflow is caught in a sterile test tube and the metal plug replaced. The plug and the end of the rubber tubing is then sterilized by dipping in boiling water for a second and at once is submerged in the water of the main tank. In this way, at intervals of time selected for the experiment, small portions of about 10 c.c. of the tuberculous milk can be withdrawn into a series of test tubes which are immediately placed in a cold water bath and cooled.

The advantages of this method of heating are worthy of special comment in view of the great irregularities of the results due to imperfect heating equipment. When the lead coil has been filled with tuberculous milk, it is closed at both ends. At the inlet end it is closed by surplus milk in the inlet pipe. At the outlet it is closed by the metal plug. The complete filling of the coil removes all air. The milk in the coil, therefore, is not in contact with outside air. As a result, the formation of pellicle, foam or splash is impossible. The submerged coil is uniformly heated, as the hot water in the bath is stirred. Therefore the milk in the coil is uniformly heated. By the movement of the milk through the coil it is also thoroughly mixed. The milk, which first fills the coil, remains at the temperature of the coil until it is withdrawn at the outlet. Therefore the period between the filling of the coil and the withdrawal of any portion of milk is the length of time the milk was heated. The amount of milk in the coil at the first filling is sufficient for all of the portions needed for a single test series.

Testing the milk. The milk was tested by the inoculation of guinea pigs. About 10 c.c. of milk was the portion withdrawn in each of the series of test tubes representing the different periods of time. These tubes, after cooling, were centrifuged for five minutes, and two guinea pigs were inoculated with one c.c. of the liquid from the top and from the bottom of each tube.

Thus the cream and the sediment of the milk in each tube was tested. The guinea pigs were kept in suitable cages for ten weeks and were then killed and examined for tubercular lesions. Any pigs dying before the expiration of that time were examined at death. Where typical tubercular lesions were found, the results were reported as positive. Where no tubercular lesions were found, they were reported as negative. Where the lesions were of doubtful character or suspicious, they were macerated in sterile salt solution and subinoculations made in additional guinea pigs.

Test series. A total of fifteen separate series of tests were made. The first three were in open vessels; the remaining 12 in the lead coil. The water tank was heated to twelve different temperatures at different times. These temperatures were: 170°, 160°, 155°, 150°, 145°, 142°, 140°, 138°, 136°, 134°, 132°, Fahrenheit. From the records of previous investigators, the approximate limits of time within which the tubercle bacillus would be killed at each temperature were known in advance. Therefore the ranges of times chosen for each temperature were selected with this information as a guide.

TABLE 1.
THERMAL DEATH CURVE OF TUBERCLE BACILLUS.

Temperature.	10 sec.	20 sec.	30 sec.	45 sec.	1 min.	2 min.	2½ min.	3 min.	4 min.	5 min.	6 min.	7½ min.	8 min.	10 min.	12 min.	12½ min.	15 min.	17½ min.	20 min.	22½ min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.	75 m in.	90 min.
212°..............	— —	— —	— —																										
200°..............	..	— —	— —																										
180°..............	..	— —	— —																										
170°..............	..	— —	— —	— —	— —																								
160°..............	..	— —	— —	..	— —	— —																							
155°..............	..	..	— —	..	— —	— —	..	— —																					
150°..............	..	..	+ +	..	+ +	— —	..	— —	— —	— —																			
145°..............	..	..	+ +	..	+ +	+ +	..	..	+ —	..	— —	..	— —	— —	+* —														
142°..............	..	..	..	..	..	..	+ +	..	..	— —	..	— +	..	— —	..	— —	— —	— —	— —										
140°..............	..	..	..	..	..	..	..	..	....	+ +	..	..	..	— —	..	..	— —	— —	— —	— +*									
138°..............	..	..	..	..	..	..	..	..	..	+ +	..	..	..	+ —	..	..	+ +	..	— —	..	— —	— —	..	— —					
136°..............	..	..	..	..	..	..	..	..	..	..	..	..	..	+ —	..	..	+ —	..	+ +	..	+ +	— —	..	— —	..	— —			
134°..............	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+ +	..	..	— +	..	— —	.. ..	— —	— —	..	..
132°..............	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+ —	..	..	+ +	..	— —	— —	— —
130	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+	..	..	..	..	—	..	—

RESULTS

The results of all of the autopsies on guinea pigs are shown in Table No. 1.

In this table the minus signs indicate the guinea pigs that were negative and the plus signs those that were positive. The results seem to be consistent in that the higher temperatures gave negative results in shorter times while the lower temperatures gave negative rsults at longer times, with the exception of the temperatures of 145° and 140°. In the case of 145° there were negative results in 6, 8, and 10 minutes, with 2 guinea pigs used at each temperature. On the other hand, at 12 minutes there was 1 positive and 1 negative result. This, however, was the last sample of milk taken from the apparatus, and it is believed that an error was committed by the operator in removing more milk than was contained in the coil at its first filling, so that fresh milk was allowed to come through which had not remained in the vessel the full period of time. In the case of the temperature of 140°, 2 negative results were obtained in 10, 15, 17½, and 20 minutes. This corresponds to the results obtained by Theobald Smith, Russell and Hastings and others at this temperature. On the other hand, at 22½ minutes one negative and one positive result occurred. This also was the last sample of milk taken from the apparatus, and as in the former case is believed to have been some fresh milk which had not remained in the coil a sufficient length of time. In this series of tests the results at other temperatures and also the results of previous investigations justify the conclusion that the positive guinea pigs from these two cases were due to errors by the laboratory worker.

If the temperatures and times at which negative results were obtained are arranged in order they give the following tabulation:

THERMAL DEATH POINT OF THE TUBERCLE BACILLUS

Temperature	Time
212° F.	10 sec.
200	20 sec.
180	20 sec.
170	20 sec.
160	20 sec.
155	30 sec.
150	2 min.
145	6 min.
142	10 min.
140	10 min.
138	20 min.
136	30 min.
134	40 min.
132	60 min.
130	60 min.

If these points are arranged on a chart in which the intervals of temperature and time are spaced regularly and are connected with a line, such line forms a remarkably smooth curve, as shown in Chart 1. This curve may be properly designated by the title "Thermal death curve of the tubercle bacillus," since it contains within itself all of the infinite number of thermal death points of this species for any temperature and any time within the limits used.

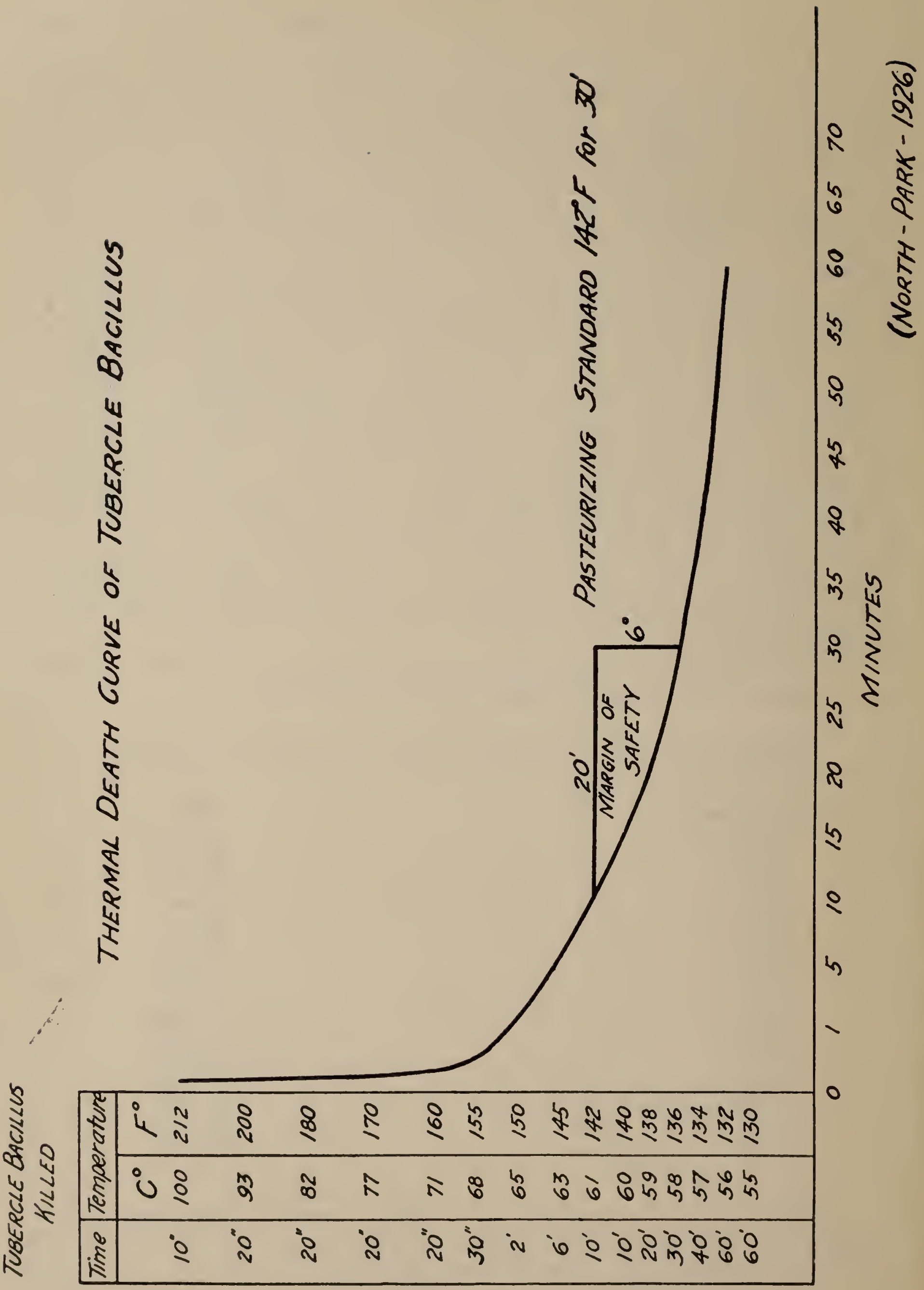

CHART No. 1

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MARGIN OF SAFETY

The chief importance to public health of an accurate determination to establish the thermal death curve of the tubercle bacillus, such as has been attempted herein, is to lay the foundation for standards for the pasteurization of milk. It is obvious that no standards for pasteurization should be made identical with any of the temperatures and times necessary to kill the tubercle bacillus, since this would make no allowance for irregularities in the operation of the process of pasteurization. The term "margin of safety" refers to the additional temperature and additional time necessary to provide an adequate margin, above any thermal death point of the tubercle bacillus, so that under no circumstances, in the routine operation of pasteurization, will the process fail to destroy the tubercle bacillus. It is obviously necessary in forming such an estimate to have reliable information as to the extent of the irregularities and fluctuations inherent in the pasteurizing process. It is to be regretted that such estimates in the past have not been based on scientific experimental data, but have been based for the most part on surmises and assumptions.

The pasteurizing apparatus, when properly made and supervised by competent operators, will confine the fluctuations in temperature within a fraction of one degree, and the period of time can be as accurately controlled as it can be under laboratory conditions. As a matter of fact, modern pasteurizing equipment, under automatic control in numerous pasteurizing plants, consistently controls both the temperature and the time even more accurately than can be done by hand operation in the laboratory. There is no margin of safety large enough to provide against the irregularities created by ignorance or dishonesty on the part of operators of such a process. If we consider, for example, the pasteurizing standard which had been adopted by New York City and fifteen other large cities, which is a minimum temperature of 142° F. for a minimum period of 30 minutes, an inspection of the chart shows that this temperature is 6° higher than the temperature of 136° at which the tubercle bacillus was killed at a period of 30 minutes in these experiments. The period of 30 minutes is 20 minutes more than is necessary to destroy the tubercle bacillus at 142° F. The city standards of 142° F. for 30 minutes then provide a margin of 6° in temperature and 20 minutes in time as a safeguard against accidental fluctuations in the pasteurizing process.

With modern equipment and competent operators the temperature can consistently be maintained between 143° and 142° F. with the time accurately controlled for 30 minutes. The 6° of temperature mentioned, therefore, provide an abundant margin of safety. Those who advocate the higher temperature of 145° for a period of 30 minutes do so on the assumption that the extra 3° is sufficient to cover the irregularities of imperfect equipment and incompetent operation. This is far from the truth since no pasteurization standard, even that of 212° is sufficient to provide against pasteurizing processes of this kind.

Another factor which has not rceived the attention it deserves has been pointed out by Professor Bang, who states that in feeding experiments with young rabbits the heating of milk for 2 minutes at a temperature of 140° was sufficient to weaken tubercle bacilli so that they could not infect rabbits through the alimentary canal.

It is obvious that there is a marked difference between the resistance of any animal to a hypodermic injection of tubercle bacilli, as compared with the feeding of tubercle bacilli. All of the tests made by hypodermic injection, as was done in these experiments, subject the laboratory animals to a far more severe strain than would be the case if the same dose of tubercle bacilli were administered with their food. The fact that the milk is not injected hypodermically into the tissues of milk consumers, but is taken by mouth, provides an additional margin of safety which is fairly indicated by the difference in resistance mentioned by Professor Bang. If, therefore, 2 minutes at 140° F. is sufficient to prevent infection when milk is introduced into the alimentary canal, a 30-minute period provides a margin of 28 minutes in time, which must be considered in estimating the margin of safety contained in any definition of pasteurization.

Conclusions

These are based both on the survey of the previous investigations and on studies of the operation of pasteurizing equipment and on the data reported herein.

1. Irregularities in the reports of various thermal death points of the tubercle bacillus are due primarily to faults in heating equipment and its operation by the investigators. These irregularities can be easily overcome by the use of simple heating equipment which can be properly operated by any intelligent operator.

2. There is no material difference in the resistance to heat between bovine and human strains of tubercle bacilli, and between different strains of either species, or between the tubercle bacilli in naturally infected milk, tissue lesions, or pure cultures.

3. Some of the work done by previous investigators was properly performed and furnished reliable data. These data indicate several thermal death points, as follows:

212° F. to 185° F. when heated for a few seconds
180° F. for 2 minutes
170° F. for 5 "
140° F. for 20 "

4. The volume of data reported in Bulletin No. 147 in the tests made at 142° F. and at 138° F. exceeds in quantity, by a large margin, the volume of data reported in any of the previous investigations, the results of which have already been accepted by bacteriologists. The care with which the work was performed and the authority of the investigators justifies the conclusions therein stated that tubercle bacilli are killed at 138° F. when heated for 30 minutes, and that a standard of 142° F. for 30 minutes is a proper standard for pasteurization.

5. There are an infinite number of thermal death points for the tubercle bacillus since for each period of time there is a degree of temperature at which this species of bacteria is destroyed. The number of tests sufficient to furnish statistical data shows that these thermal death points range themselves in regular order, the whole series, when plotted on a chart, forming a regular curve. This time-temperature relationship may be properly described as a "thermal death curve" rather than a "thermal death point."

6 If milk consumers were inoculated with hypodermic needles, as is done to guinea pigs, then the margin of safety of a pasteurizing standard of 142° F. for 30 minutes would provide for an excess of 6° in temperature and 20 minutes in time. In addition to this margin of safety it must be

recognized that milk consumers possess within themselves an additional safeguard against infections administered by the intestinal canal.

7. Temperature standards for pasteurization which injure the milk are not necessary for the protection of public health. The standard of 145° F., advocated by some authorities, changes the appearance of milk and thereby discredits pasteurization in the minds of milk consumers. It is in the interest of public health that pasteurization should be everywhere accepted. Its acceptance is prevented by such milk injuries. Standards used by New York City and fifteen other large cities for more than ten years have been proved by the experience of the health authorities of these cities satisfactory safeguards over public health, and at the same time have not injured the milk in a way that would prevent its acceptance by the public. These standards are a minimum temperature of 142° F. for a minimum time of 30 minutes. The results of the researches of previous investigators and of the researches herein reported show that this pasteurizing standard provides an adequate margin of safety.

For bibliography see the original article.

FURTHER STUDIES UPON THE COMPLEMENT FIXATION TEST IN CHRONIC GONORRHEA IN WOMEN

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M. A. Wilson, Mary V. Forbes and Florence Schwartz

These studies were undertaken in 1919 in coöperation with the Division of Venereal Diseases of the New York State Department of Health with the purpose of determining the value of the complement fixation test, as compared with other laboratory examinations, for the diagnosis of gonorrhea in women. The development of a clinical classification and improvements in method for smear, culture and complement fixation diagnoses were accomplished during the first year. The clinical classification and a comparison of diagnoses by smear, culture and complement fixation in 50 cases of mild, chronic gonorrhea in women were given in our preliminary report. Since then the time has been devoted to attempts to improve further the technic of the complement fixation test and to obtain an answer to the following questions: "Is the gonococcus complement fixation test specific?" "Is it an aid to the clinician in diagnosis?"

In attempting to answer the first question it is necessary to take up in detail the various factors in the test and, in discussing the possible defects, to show also the efforts that have been made to overcome some of these defects.

Although the second question is, in part, dealt with by tables 3 and 4, it is answered more fully in clinical papers published by Emily Dunning Barringer and E. von Bose and by Barringer and Williams.

Experiments to Improve the Gonococcus Complement Fixation Test

The technic of our diagnostic test was described briefly in our preliminary report (1) and later a detailed account of the method with interpretations of all the reactions was published in Pathogenic Microörganisms by Park and Williams. This method had been adopted by us as a standard before the present study was begun and has been continued as a standard, or control, in our attempts to improve the test.

Our experiments include:

Investigation of various methods for the preparation of antigen.

Tests as to the anticomplementary reaction of the patient's serum and the presence of natural antisheep amboceptor.

Standardization of complement.

Antigen

Many experiments have been undertaken by us to develop an antigen that will give a higher percentage of positive complement fixation reactions with serums of gonorrheal patients. These experiments dealt with the antigenicity of the gonococcus, the advantage of polyvalent antigens and the comparison of various culture media for antigen production.

Comparison of the antigenicity of the Torrey strains with recently isolated gonococci

In 1916 Dr. Archibald McNeil invited our coöperation in his study of antigen preparations. We isolated gonococci from 28 cases of acute urethritis in the male and made antigens of the new cultures at the same time and upon the same medium as the antigens prepared with the Torrey strains. Comparative tests of all the antigens were made with gonococcus immune rabbit serums and human gonorrheal serums. The freshly isolated cultures proved to be of lower antigenicity than the Torrey strains when tested with the same serums. The antigens from freshly isolated cultures had to be used in 1:10 dilution to obtain the same reactions that occurred with the Torrey strains in 1:20 dilution. The new isolations have less growth in the forty-eight hours than the Torrey strains, but the suspensions of the new cultures before being diluted and the suspensions of the Torrey strains before dilution were macroscopically of the same density, and all the antigen suspensions were made by weighing the dried cocci and adding saline in the proportion of 1 gram of dried cocci to 200 cc. of saline solution.

At the beginning of our present study, Miss Kutner isolated gonococci from 10 cases of gonorrhea and prepared an antigen from the cultures. These cases included both male and female adults.

Our complement fixation tests with her antigen were similar to those obtained with our antigens made from freshly isolated cultures.

In 1920 we isolated gonococci from 42 cases of gonorrheal vaginitis and made several polyvalent antigens. These, also, gave the same reactions in 1:10 dilution that the Torrey strain antigens gave in 1:20 dilution, thus, confirming the results of our two previous studies.

These experiments convinced us that the Torrey strains had not lost their antigenicity, and we have continued to use them for our stock antigens.

Will autogenous antigens and other monovalent antigens detect gonococcus antibodies that are missed by the Torrey strains?

Up to the present time we have given very little study to this subject, but are planning to follow it more fully in the future.

We have made three monovalent antigens: one from a culture isolated from acute gonorrheal vaginitis (Pecchio), one from a gonococcus isolated from Bartholin's gland (Dolson), and one from the blood of a scarlet fever patient having the complication of gonorrhea (Fazzio). The Dolson and Fazzio cultures were identified as gonococci by positive agglutination reactions in high dilution.

The agglutination tests were made by Dr. John C. Torrey.

The Pecchio antigen did not give a complement fixation reaction with the Pecchio human serum, but gave complete fixation, in 1:10 dilution, with a polyvalent serum from a rabbit immunized with the ten Torrey strains. The Pecchio human serum gave a weakly positive reaction with the Torrey polyvalent antigen. The Dolson monovalent antigen gave the same weakly positive reaction with its homologous serum as that serum gave with the Torrey polyvalent antigen and with the Fazzio monovalent antigen. These tests were made at the same time and with the same reagents. There was insufficient antigen prepared to continue the studies and the cultures were lost before another lot of antigen could be prepared. The following table shows these reactions:

TABLE 1.

COMPLEMENT FIXATION WITH MONOVALENT AND POLYVALENT ANTIGENS

Serums	Antigens			
	Monovalent			Polyvalent
	Pecchio dilution 1:10	Dolson dilution 1:10	Fazzio dilution 1:10	Torrey strains dilution 1:20
Pecchio (human)	Negative	No test	No test	Weakly positive
Dolson (human)	No test	Weakly positive	Weakly positive	Weakly positive
Fazzio (human)	No test	Strongly positive	Strongly positive	Strongly positive
Control. Rabbit immunized with Torrey's ten gonococcus strains	Strongly positive	Strongly positive	Strongly positive	Strongly positive

In the above tests it will be seen that the Pecchio serum did not react with its own antigen, but did react with the Torrey antigen.

The Dolson and Fazzio serums gave the same reactions with their own antigens that they did with the Torrey antigen. The rabbit serum (Torrey strains) reacted with all the antigens.

Effects of culture media upon antigenicity of the gonococcus

We have made polyvalent antigens with the ten Torrey strains upon various media, including salt-free-veal-agar, Vedder's starch medium, glucose-ascitic-veal-agar, vitamin-agar, glycerine-horse-serum-veal-agar, and disodium-phosphate-agar. Our findings are that the antigenicity of the gonococcus is not affected by the culture medium if the medium gives an abundant growth within forty-eight hours. Antigens made from twenty-four-hour, or seventy-two-hour growths, have to be used in lower dilution than those made from forty-eight-hour growths. However, although the antigenicity of the gonococcus is not affected by the culture medium, its anticomplementary property may be increased. We have found the antigens made from growth on Vedder's starch medium to be anticomplementary. Iodin tests revealed starch granules after the cocci had been washed many times to free them from the starch.[1] Media containing hemoglobin, also tend to produce an anticomplementary antigen. We have had uniformly good results with antigens made from growth on glycerine-horse-serum-veal-agar and on disodium-phosphate-agar. A small volume of antigen from growth on wheat medium was tried. There was not enough of the wheat medium antigen to test the human serums, but this antigen gave the same reaction with the gonococcus immune-rabbit-serum that the disodium-phosphate-agar antigen did.

We are planning a more complete study of different methods of antigen production.

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[1] The tests for starch were made by Dr. McNeil.

THE PATIENT'S SERUM

Two factors tend to give "false" reactions with the patient's serum: the anticomplementary reaction of the serum and the presence of natural antisheep amboceptor. The former gives a so-called false positive reaction, and the latter a false negative reaction.

Anticomplementary serums

In this laboratory we have had less than 1 per cent of anticomplementary patients' serums that could not be controlled by heating. The most notable example was a serum from a sixteen-year-old girl whose clinical diagnosis was "acute gonorrhea." This patient was bled at intervals of one week for thirteen weeks. The bleedings were tested in two laboratories and all reports were returned as "anticomplementary."

We tried a number of experiments to overcome this anticomplementary property. Upon the theory that the anticomplementary reaction in the patient's serum is due to the presence of both antigen and antibody in the right proportion to absorb complement, we tried to break down the antigen-antibody combination by adding either an excess of antigen, or an excess of antibody, but none of our experiments eliminated the anticomplementary property.

Active and inactivated serums gave the same anticomplementary reaction. The simple method recommended by Breuer was not tried on this serum, as his article had not been published at that time and his procedure had not been thought of by us. Breuer used a constant volume of patient's serum with varying amounts of complement and found that he could always obtain some dose of complement that would enable him to make a diagnosis of serums that would be anticomplementary with the usual dose of complement.

THE PRESENCE OF NATURAL ANTISHEEP AMBOCEPTOR IN THE PATIENT'S SERUM

The question of natural antisheep amboceptor is one to be considered. In table 2 are tabulated the results in a study of 2374 bleedings from 753 patients. The incidence of natural antisheep amboceptor that gave a negative reaction in a positive serum was only 0.3 per cent. In 60.6 per cent of these 2374 bleedings there was enough natural antisheep amboceptor to hemolyze from one-fourth to three-fourths of the sheep cells in the hemolytic system. In 50 per cent of the bleedings represented by the 60.6 per cent the small content of natural antisheep amboceptor did not interfere, apparently, with the complement fixation reaction. This conclusion was drawn from the fact that a human bleeding of one week might have no natural antisheep amboceptor and would give a weakly-positive reaction, while on the following week a bleeding from the same patient might have enough natural antisheep amboceptor to hemolyze half the cells and continue the weakly-positive reaction.

On the other hand, a number of serums having enough natural amboceptor completely to hemolyze all the cells in the hemolytic system have given a strongly positive reaction. In a recent example of this occurrence, the diagnostic test continued to be four-plus after standing in the water-bath for one hour, and then overnight in the ice-box.

As a control for natural antisheep amboceptor, we have used the Kaliski modification of the Bauer test, in which the natural antisheep amboceptor in the patients' serum is utilized for completing the hemolytic system, and, in the event of there being no antisheep amboceptor, or less than one unit, enough artificial amboceptor is added to produce complete hemolysis in the hemolytic system.

We have found the Kaliski method to be inadequate as a diagnostic test for the reason that a completely hemolyzed system is seldom obtained when artificial amboceptor is added to supplement the natural antisheep amboceptor.

The nature of the natural antisheep amboceptor reaction has not been explained sufficiently to enable one to control it absolutely. That an antigen in association with its homologous antibody can be dissociated from that antibody by the addition of an excess of artificial antisheep amboceptor is illustrated by the following example:

DISSOCIATION OF ANTIGEN FROM ITS HOMOLOGOUS ANTIBODY

First period of the test

Reagents	Diagnostic Test				Kaliski-Bauer Control			
	Serum control		Serum+antigen		Serum control		Serum+antigen	
Serum	0.04	0.02	0.02	0.01	0.04	0.02	0.02	0.01
Antigen	None	None	0.1	0.1	None	None	0.1	0.1
Complement	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Fixation period: One hour in water-bath at 37°C.

The foregoing chart shows the contents of all the tubes in the complement fixation tests up to the end of the fixation period. It will be noticed that the diagnostic test and the Kaliski-Bauer conrol contain exactly the same reagents in the same amounts. They are both fixed at the same temperature for the same time. Then, to the diagnostic test is added the standard dose of sensitized cells. (This dose contains 0.1 cc. of a 5 per cent suspension of sheep cells and two hemolytic units of antisheep amboceptor.) To the Kaliski-Bauer control is added 0.1 cc. of a 5 per cent suspension of sheep cells without any artificial amboceptor. After the cells have been added to all the tubes, the tests are replaced in the water-bath for the second incubation and at the end of ten minutes the reactions are read. The following chart shows the reactions in the tubes at the end of the second incubation period, after the addition of sensitized cells to the diagnostic test and 5 per cent suspension to the Kaliski-Bauer control:

Second period of the test After ten minutes in water-bath

	Diagnostic Test		Kaliski-Bauer Control	
	Serum control	Serum + antigen	Serum control	Serum + antigen
Reaction	Completely hemolyzed	Completely hemolyzed	Completely hemolyzed	Completely fixed
Diagnosis	Negative		Four-plus Interpreted as strongly positive	

As the above test and control were identical up to the end of the fixation period, if the complement were fixed as shown in the reading of the Kaliski-Bauer control, it must, also, have been fixed in the diagnostic test. Therefore, we conclude that the presence of natural antisheep amboceptor in the patient's serum did not interfere with the fixation of complement, but, that the addition of an excess of antisheep amboceptor dissociated the gonococcus fixation amboceptor in the patient's serum from the gonococcus antigen, thus, liberating the complement that had been completely fixed and giving a negative reaction. This point continues to be a subject for study in our laboratory.

The absorption of natural antisheep amboceptor has been recommended by some writers, but we have not found it to be an adequate remedy.

Table 2 shows the incidence of natural antisheep amboceptor in 2374 bleedings.

TABLE 2.

SHOWING THE INCIDENCE OF NATURAL ANTISHEEP AMBOCEPTOR IN PATIENTS' SERUMS

Number of Bleedings	Percentage Having One Unit, or More, of Natural Antisheep Amboceptor			Percentage Having less than one unit of natural antisheep-amboceptor	Percentage having no natural antisheep-amboceptor
	1.3				
	These are subdivided into:				
	Per cent positive reactions reduced to negative	Per cent positive reactions not changed	Per cent doubtful reactions		
2374	0.3	0.1	0.9	60.6	38.1

COMPLEMENT

The source of greatest difficulty in the performance of the complement fixation test lies in the variation of the guinea-pig complement. As we have shown in our preliminary report, the guinea-pig serums vary in fixability as well as in hemolytic activity, and before being pooled for tests all guinea-pig serums must be tested for natural antisheep amboceptor, hemolytic activity, anticomplementary reaction with the gonococcus antigen, anticomplementary reaction with the control gonococcus serum, and for fixability with the gonococcus antigen-serum-complex. The details of these preliminary tests and their interpretation have been published.

During the year 1920, out of 481 guinea-pigs tested, only 57 per cent could be used for gonococcus complement fixation.

HEMOLYTIC SYSTEM

After selecting the complement by the preliminary tests, the pooled complement is titrated with sensitized cells in order to determine the exact dose of pooled complement to be used for tests.

The sensitized cell dose contains two hemolytic units of antisheep amboceptor and 0.1 cc. of 5 per cent suspension of sheep cells.

The complement unit is read at the end of thirty minutes in the water-bath at 37° C. We use two hemolytic units of complement for diagnostic tests.

THE DIAGNOSTIC TEST

We use one-tenth the original Wassermann volumes of all reagents.

The diagnostic tests are made in duplicate, with controls for the hemolytic system, for anticomplementary reaction of the patient's serum, for natural antisheep amboceptor in the patient's serum, and for fixation unit and anticomplementary reaction of the gonococcus antigen.

The diagnostic test and all controls are fixed in the water-bath for one hour at 37°C.

Reading. The following controls must be completely hemolyzed before the diagnostic tests are read: system control, control for anticomplementary reaction in patient's serum, the antigen anticomplementary control (0.4 cc. of the antigen dilution must be completely hemolyzed, that is, four times the dose used in the test).

The average complement used throughout this study has given complete hemolysis in all controls in twelve minutes.

Diagnosis. If the patient's serum contains no natural antisheep amboceptor and if it is not anticomplementary, the diagnosis is made in accordance with the reactions in the tubes containing antigen plus serum. The Citron method for readings is used, with the exception of our "strong-one-plus," "one-plus" and "plus-minus" reactions, which Citron does not mention in his description of reactions.

Our "strong-one-plus" has very strong fixation in both the 0.02 and 0.01 tubes of the test; our "one-plus" has strong fixation in the 0.02 and weak fixation in the 0.01; our "plus-minus" has partial fixation in both the 0.02 and 0.01 tubes.

Anticomplementary serums. If the control for anticomplementary reaction in the patient's serum is not completely hemolyzed at the time required for hemolysis of the other hemolytic controls, the test is replaced in the water-bath until the end of one hour. If at that time the serum still shows any degree of anticomplementary reaction, no diagnosis is made of that bleeding, and it is reported as "anticomplementary."

Natural antisheep-amboceptor. If the diagnostic test is negative and the Kaliski-Bauer control shows a typical four-plus reaction, we report the test as: "Four plus by Kaliski-Bauer control." If the diagnostic test is negative and the Kaliski-Bauer control shows less than a four-plus reaction, we report the test as: "Negative. Serum contains an excess of natural antisheep amboceptor." In such cases we request another bleeding. If the serum controls in the Kaliski-Bauer control have partial hemolysis, we report the test according to the diagnostic test, regardless of the small amount of natural antisheep amboceptor. In table 2 we have shown that the presence of natural antisheep amboceptor reduced a positive reaction to negative in only 0.3 per cent of 2374 bleedings. Although this seems an insignificant number, we continue to use the control for the sake of the individual, and to give us an explanation of variation in the complement fixation curves of patients bled week after week.

Interpretation of diagnosis. All tests showing complete fixation in the 0.01 tube are interpreted as "very strongly positive." This is the Citron "four-plus" reaction, and is his interpretation.

All tests showing strong fixation in the 0.01 tube are interpreted as strongly positive. This includes Citron's "three-plus" and our "strong one-plus" reactions, and is Citron's interpretation of his "three-plus." In our "strong one-plus" reaction the 0.02 and 0.01 tubes are both very strongly fixed. Citron does not mention this reaction, but it is not infrequently encountered in the tests in this laboratory and must be considered in an accurate interpretation of diagnoses. It is stronger than Citron's "two-plus" and a shade less (in the 0.02 tube only) than Citron's "three-plus."[2]

All tests showing strong fixation in the 0.02 tube and weaker fixation in the 0.01 tube are interpreted as moderately positive. This is our "one-plus" reaction. It is stronger in the 0.01 tube than Citron's "two-plus," and a shade less in the 0.02 tube than his "two-plus." In our interpretation of moderately positive reactions, we include the Citron "two-plus." The latter is infrequently encountered in our tests, while our "one-plus" is of frequent occurrence. Citron does not describe our "one-plus."

All tests in which the 0.02 and the 0.01 tubes show partial fixation, (from 25 to 50 per cent hemolysis), are interpreted as weakly positive. This is our "plus-minus" reaction. It is stronger in the 0.01 tube than Citron's "two-plus" and has the same degree of fixation in the 0.02 tube as his "one-plus." This reaction is not described by Citron, but is of frequent occurrence in our tests.

In our weakly-positive interpretation we include Citron's "one-plus."

All tests showing 75 per cent. or more hemolysis in the 0.02 tube are interpreted as "doubtful."

Our interpretation is the result of the study of a large number of tests in relation to the clinical classification.

In cases clinically diagnosed as negative for gonococcus we have obtained no fixation, not even a doubtful reaction, and for that reason we feel justified in placing in the positive column the reaction described above.

SPECIFICITY OF THE GONOCOCCUS COMPLEMENT FIXATION TEST

In this study we have examined repeated bleedings from 870 gonorrheal cases controlled by bleedings from 350 non-gonorrheal individuals. In testing the specificity of the gonococcus antibody 92 of the gonorrheal bleedings were tested with tuberculosis antigen and gave negative reactions, and 54 of the gonorrheal bleedings were tested with *Streptococcus viridans* antigen and gave negative reactions. We have not included the Wassermann antigen in our controls of specificity of antibody for the reason that a considerable number of the patients in this service have a clinical diagnosis of syphilis, or doubtful for syphilis, as well as of gonorrhea.

In testing the specificity of the gonococcus antigen, bleedings from 350 patients clinically diagnosed as non-gonorrheal gave no trace of gonococcus complement fixation reaction.

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[2] Unpublished report of the Research Laboratory Committee on Standardization of the Wassermann Test.

Table 3 shows the incidence of gonococcus complement fixation at various stages of the disease and also the specificity tests upon non-gonorrheal serums

TABLE 3

GONOCOCCUS COMPLEMENT FIXATION IN VARIOUS STAGES OF GONORRHOEA IN WOMEN

CLINICAL DIAGNOSIS	NUMBER OF CASES	GONOCOCCUS COMPLEMENT FIXATION			
		Positive	Negative	Doubtful	Percentage positive
Acute gonorrhea.*	19	9	6	4	47
Subacute gonorrhea	112	54	36	22	48
Chronic gonorrhea	383	283	56	44	73.6
Syphilis and gonorrhea	162	132	14	16	81.4
Syphilis, doubtful for gonorrhea	113	24	88	1	21
Doubtful gonorrhea	81	49	21	11	60
Controls					
Adults having no symptoms or history of Gonorrhea	50	0	50	0	0
Children: Scarlet fever patients having no symptoms or history of gonorrhea	36	0	36	0	0
Spinal fluids from poliomyelitis cases	12	0	12	0	0
Adults: Tubercular patients having no gonorrheal symptoms	248	0	248	0	0
Normal bleedings from workers in this laboratory	4	0	4	0	0
Total cases	1220				

*Not *early* acute.

Table 4 shows the comparison of smear, culture and complement fixation in 181 cases. These three laboratory tests are more fully discussed in a paper by Torrey and Wilson.

TABLE 4.

COMPARISON OF DIAGNOSES BY SMEAR, CULTURE AND COMPLEMENT FIXATION

CLINICAL DIAGNOSIS	NUMBER OF CASES	GONOCOCCUS COMPLEMENT FIXATION											
		Smear positive, culture positive			Smear doubtful, culture positive			Smear doubtful, culture negative			Smear negative, culture negative		
		Positive	Negative	Doubtful	Positive	Negative	Doubtful	Positive	Negative	Doubtful	Positive	Negative	Doubtful
Acute	11	3	2	1	1			1	2				1
Subacute	43	10	1	3	6	1		9	4	3		4	2
Chronic	85	9	1	1	2			39	2	8	15	2	6
Chronic and syphilis	35	3		1				16	2	5	7	1	
Doubtful	7							2	3		1	1	
Total cases	181												

In table 5 is given a summary of the diagnoses reported in table 4, showing how the percentage of positive diagnoses by smear and culture decrease with the stage of the disease and how the percentage of positive complement fixation increases.

TABLE 5.

SUMMARY OF DIAGNOSES

Clinical Diagnosis	Number of Cases	Percentage of Positive Diagnosis		
		Smear	Culture	Complement fixation
Acute gonorrhea*........................	11	55	55	45
Subacute gonorrhea......................	45	32	44	58
Chronic gonorrhea.......................	85	13	15	76
Gonorrhea and syphilis..................	35	11	11	76
Doubtful gonorrhea......................	7	None	None	42
Total cases.............................	181			

* Not *early* acute.

SUMMARY

Investigation of various media for the preparation of gonococcus antigen showed that any of the starch-free and hemoglobin-free media that would give a profuse growth of the gonococcus within forty-eight hours at 37°C. would give antigens of equal value.

Recently isolated gonococci were of lower antigenicity than the Torrey strains isolated many years ago.

Autogenous antigens gave no stronger reactions with their homologous serums than did the Torrey strain antigen.

In this study we have encountered very few anticomplementary patients' serums that could not be controlled by heating.

The incidence of natural antisheep amboceptor that gave a negative reaction in a positive serum was only 0.3 per cent of 2374 bleedings. We found the Kaliski-Bauer test to be inadequate as a diagnostic measure, but of value as a control. Absorption of natural antisheep amboceptor did not give us uniform reactions.

As previously reported, we found the difference in fixative and hemolytic value of guinea-pig complements to be the greatest factor of variation in the gonococcus complement fixation test.

In order to give a true interpretation of our diagnostic tests in relation to the clinical classification, we have had to include three reactions frequently encountered in our tests that were not mentioned by Citron. Otherwise, our interpretations agree with Citron's.

Specificity tests with heterologous antigens gave no fixation with gonorrheal serums, as shown in table 3.

Non-gonorrheal serums tested with gonococcus antigens gave no complement fixation.

Tables 4 and 5 show the relative value of smear, culture and complement fixation. These three laboratory methods are discussed in a paper by Torrey and Wilson.

The clinical value of our gonococcus complement fixation tests is discussed in the publication of Barringer and Williams, and Barringer and von Bose.

CONCLUSIONS

1. Individual guinea-pig-serums must be tested for fixability with known gonococcus antibody plus gonococcus antigen before they can be used as complement for tests of patients' serums.

2. The negative results with control human serums throughout this series of tests indicate that the gonococcus complement fixation tests is specific.

3. The gonococcus complement fixation test is an aid to the clinician in institutional work.

For bibliography see the original article.

A STUDY OF THE KAHN PRECIPITATION METHOD FOR THE SERUM DIAGNOSIS OF SYPHILIS

Published in The American Journal of Syphilis, 1928, Vol. XII, pp. 111-121.

By M. A. Wilson, Rose M. Nedley and Florence Honig

This study covers a period of two years, during which time 22,030 serums (3,424 cases) were tested by the Kahn precipitation test in comparison with the Wassermann test.

The authors desire to express their appreciation of the cooperation in this investigation of Dr. Louis Chargin, Chief of the Division of Venereal Diseases, who selected cases for our study; of Mr. John Koopman, Bacteriologist in charge of the Wassermann Laboratory, who gave us serums for Kahn tests and reports of his Wassermann diagnoses of those serums, as well as giving us space for three technicians in his laboratory; of Dr. David Kalisky, of Mt. Sinai Hospital, who sent us serums, clinical data, reports of his Wassermann tests, and also sent his technician to our laboratory to make comparison tests and readings with our technicians.

We are particularly indebted to Dr. E. von Bose, of Kingston Avenue Hospital, for her kindness in sending us clinical data which enabled us to make classified tables for our report.

The precipitation tests were made by Kahns' latest method.[1] Dr. Kahn very kindly gave us personal instructions in regard to all the technic of the test.

Mr. Koopman's Wassermann tests were made with the crude alcoholic antigen, only, by the New York City Department of Health qualitative method.[2]

In the series of serums sent from the Venereal Disease Clinics, the results were summarized, as follows:

Medical Advice Clinic. 118 cases, one bleeding from each case. Agreement in diagnosis 85%.

By the term, "agreement in diagnosis," is meant that both tests were positive (i. e., 4, 3, or 2 plus), or doubtful, (+), or negative.

This term corresponds to Kahn's term, "absolute agreement," as used in his latest publication.[3]

Advice Clinic. 1503 cases, one bleeding from each case. Agreement in diagnosis, 85.5%.

Manhattan Clinic. (Treatment Cases.) 915 cases having repeated bleedings.

The *case diagnosis* (i.e., the curve of repeated bleedings) by Kahn test and Wassermann test agreed in 49% of the cases. Variation in *case diagnosis*, 51% of the cases. Two thirds of the variations can be explained as occurring at the beginning or end of the serologic test curve. These variations ranged in degree of reaction from $\left\{\begin{array}{l}\text{Kahn four-plus}\\ \text{Wassermann negative}\end{array}\right.$ to $\left\}\begin{array}{l}\text{Kahn negative}\\ \text{Wassermann four-plus}\end{array}\right.$

In Table I we have shown several examples of such variations.

TABLE 1.

Serum Number	Comparative Tests.					Explanation of Variations in the Wassermann and Kahn Tests
1	Wass.	—	—			Beginning of complement fixation curve.
	Kahn	3	4			
2	Wass.	—	—	—		" " " " "
	Kahn	±	±	2		
3	Wass.	—	—	—		" " " " "
	Kahn	±	+	3		
4	Wass.	—	±			" " " " "
	Kahn	+	2			
5	Wass.	—	—	±		" " " " "
	Kahn	±	—	+		
6	Wass.	—	±	—	±	End of complement fixation curve.
	Kahn	2	+	+	±	
7	Wass.	±	—	—		" " " " "
	Kahn	2	±	—		
8	Wass.	±	—			" " " " "
	Kahn	2	+			
9	Wass.	—	—	—		" " " " "
	Kahn	4	4	2		
10	Wass.	—	—	—		" " " " "
	Kahn	3	2	+		
11	Wass.	—	—	—		" " " " "
	Kahn	4	2	+		
12	Wass.	±	—	±	—	" " " " "
	Kahn	4	4	3	+	

For the remaining one-third of the variations in case diagnosis we find no explanation in these tests in the absence of clinical data.

As we were given no clinical diagnoses for the clinic cases, we are unable to make classified charts of those tests for this paper.

From Kingston Avenue Hospital we received blood serums from 788 cases, with a total of 3,359 repeated bleedings. Most of the bleedings were made at intervals of one week during the period one patient was in the hospital. Those tests may be summarized, as follows:

Those cases diagnosed as clinical syphilis had complete agreement in the *case diagnosis* by Kahn and Wassermann tests. By the term, "case diagnosis" is meant that throughout the curve of repeated bleedings from the patient one or more of the bleedings had the same diagnosis by Kahn and Wassermann. Under *Positive* case diagnosis are listed all the serums that were four-plus, three-plus, or two-plus by Kahn and Wassermann. Under *doubtful* case diagnosis are listed all serums that were one plus by Wassermann and Kahn. Under *negative* case diagnosis are listed all serums that were negative, or plus-minus, by Wassermann and Kahn. We placed the plus-minus reactions with the negatives instead of with the doubtfuls for the reason that usually the clinician interprets a plus-minus reaction as negative.

In cases clinically diagnosed as "Serologic Syphilis," or as "Syphilis and Gonorrhoea," there were variations in the case diagnosis by Kahn test and Wassermann test, as follows:

Serologic Syphilis:
39 cases, agreement in case diagnosis 70%.
Variations: Kahn stronger than Wasserman 20%.
Wassermann stronger than Kahn 10%.

By "Serologic Syphilis" is meant cases having no clinical symptoms of syphilis, but having a positive Wassermann test when admitted to the hospital.

Syphilis (not classified as to stage of disease) 213 cases. 185 of these were complicated with gonorrhoea and had agreement in case diagnosis in 95.5%, and the remaining 28 cases, not complicated with gonorrhoea had agreement of 57%.

Gonorrhoea, subacute:
24 cases, agreement in case diagnosis 91.5%.
Both tests positive 8.5% of the cases.
Both tests negative 83% of the cases.
Total variations: Kahn stronger in 8.5% of the cases.

Gonorrhoea, chronic:
130 cases, agreement in case diagnosis 93.5%.
Both negative 88%.
Both positive 5.5%.
Variations: Kahn stronger, 5.5%.
Wassermann stronger, 1%.

Gonorrhoea, clinical:
145 cases, agreement 88%.
Both negative 78.5%.
Both positive 9%.
Both doubtful 0.5%.
Variations: Kahn stronger, 7%.
Wassermann stronger 5%.

By "gonorrhoea clinical" is meant cases having clinical symptoms of gonorrhoea and all smears negative.

The data from which the above summary was made are shown in Table II.

TABLE 2.

SHOWING AGREEMENT OF KAHN AND WASSERMANN CASE DIAGNOSES AND RELATION OF THOSE CASE DIAGNOSES TO THE CLINICAL DIAGNOSIS.

Clinical Diagnosis	Total Cases	Percentage of Agreement of Kahn and Wassermann Diagnoses	Agreement.			Variation	
			Kahn and Wassermann Diagnosis Agreed Throughout Curve, or at some time during the Curve.			Wass. Stronger than Kahn	Kahn Stronger than Wass.
			Number of Cases Whose Case Diagnoses were:				
			Pos.	Neg.	Doubt.		
Syphilis, Primary	1	100%	1				
Syphilis, Active Secondary ..	7	100%	6	1			
Syphilis, Non-Active	7	100%	6	1			
Syphilis, Serologic	39	70%	24	3		3	9
Syphilis, Not Classified as to Stage of Disease...	28	57%	16			1	11
Syphilis and Gonorrhoea	185	95.5%	159	19			15
Gonorrhoea, Subacute	24	91.5%	2	20			2
Gonorrhoea, Chronic	130	93.5%	7	115		1	7
Gonorrhoea, Clinical	145	88%	13	114	1	6	10

NOTE: 198 cases of gonorrhoea, not classified as to stage of disease, and 22 cases from which no clinical data were received, are not included in this table.

In order to determine the exact agreement of Wassermann and Kahn tests of individual serums, the tests described as case-diagnosis, or "curve-diagnoses" in Table 2, were listed to show the number of bleedings that were four-plus by both Wassermann and Kahn, three-plus by both, etc. This list is shown in Table 3.

Table 3 is summarized as follows:

Primary syphilis, 1 case, had 6 bleedings, as follows:
    1 bleeding was plus-minus by both tests.
    1 was four-plus by Wassermann and three-plus by Kahn
    3 were four-plus by Wassermann and two-plus by Kahn
    1 was two-plus by Wassermann and negative by Kahn

Syphilis:
    Active secondary, 7 cases, 31 bleedings.
    16 bleedings were four-plus both tests
    2 bleedings were negative both tests

Syphilis:
    Non-Active, 7 cases 23 bleedings
    6 bleedings were four-plus both tests
    4 bleedings were negative both tests

Syphilis, Serologic:
    39 cases, 172 bleedings
    61 bleedings were four-plus both tests
    5 were three-plus both tests
    4 were two-plus both tests
    1 was plus both tests
    1 was plus-minus both tests
    10 were negative both tests

Gonorrhoea subacute:
    22 cases, 105 tests
    4 bleedings were four-plus both tests
    97 bleedings were negative both tests.

Gonorrhoea chronic:
    130 cases, 557 bleedings
    10 bleedings were four-plus both tests
    2 bleedings were two-plus both tests
    1 bleeding was plus-minus both tests
    515 bleedings were negative both tests

By comparing the arrangements of the same tests in Tables II and III it is shown that the percentage of agreement of the two tests will be smaller when the comparison is made upon the identical diagnosis of the same bleeding by both tests, than when the comparison is made upon the interpretations positive, negative and doubtful.

We have not found in the literature any report of a study of repeated bleedings from the same patient, and we thought it might be of interest to include in our publication a table showing the Kahn and Wassermann curves of repeated bleedings. From the total 788 cases, we have selected from each clinical classification a few curves that are fairly representative of the whole. These are shown in Table IV under the heading, "Comparative Tests," each column represents a specimen of blood tested by Wassermann and Kahn methods. The numerals in each column represent the diagnosis reported to the clinician expressed in terms of 4, 3 or 2 plus, etc.

TABLE 4

Clinical Diagnosis	Case Number	Test No.	Comparative Tests.												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Syphilis. Primary	1	Wass.	4	4	4	4	±	2							
		Kahn	2	2	3	2	±	—							
Syphilis. Active......		Wass.	—	±											
Secondary	2	Kahn.	—	—											
	3	Wass.	4	4	4										
		Kahn.	4	4	4										
	4	Wass.	2	4	4	+	±								
		Kahn.	4	4	4	4	3								
	5	Wass.	4	4	3	+	—	+							
		Kahn.	4	4	4	4	4	4							
	6	Wass. Kahn.	4 3	4 4	4 4	4 4	4 4								
	7	Wass.	4	4	4	4	±	+	+						
		Kahn.	4	4	4	4	4	4	4						
	8	Wass.	4	±	—										
		Kahn.	4	4	3										
Syphilis. Latent.	9	Wass.	4	4	2	±									
Act. Sec.		Kahn.	4	4	4	4									
	10	Wass.	+	±	+	—	2	—							
		Kahn.	3	4	4	4	4	2							
	11	Wass.	4	4	4										
		Kahn.	3	3	3										
Syphilis. Serologic.	12	Wass.	4	4	4	3	4	4	4	4	4	3			
		Kahn.	4	4	4	4	4	4	4	4	4	4			
	13	Wass.	4	4	4	4	2	—							
		Kahn.	4	3	4	4	4	4							
	14	Wass.	4	4	4	±	3	+							
		Kahn.	2	4	4	4	3	4							
	15	Wass.	2	—	—	—	2	2							
		Kahn.	2	+	+	+	2	—							
	16	Wass.	4	±	+	—	—								
		Kahn.	3	3	±	2	3								
Syphilis and Gonorrhoea	17	Wass.	—	—	—	2	4	3	2	4	4	3	+	±	±
		Kahn.	—	—	±	3	4	4	2	4	3	4	4	4	4
	18	Wass.	±	—	—	+	2	±	—	—	2	—	±	2	+
		Kahn.	—	3	2	2	3	4	2	2	2	2	2	3	2
	19	Wass.	4	4	4	4	4	4	4	4	+	±	+		
		Kahn.	3	3	+	3	4	4	3	4	±	4	4		
Gonorrhoea. Subacute	20	Wass.	—	—	—	—	—	—	—	—	—	—	—	—	
		Kahn.	—	—	—	—	—	—	—	—	—	—	—	—	
	21	Wass.	—	—	—	—	—	—	—	—	—	—	—	—	
Chronic		Kahn.	—	—	—	—	—	—	—	—	—	—	—	—	
Chronic	22	Wass.	—	—	—	—	—	—	—	—	—	—	—	—	—
		Kahn.	—	±	—	—	—	—	—	—	—	—	—	—	—
Clinical	23	Wass.	—	—	—	—	—	—	—	—					
		Kahn.	—	—	—	—	—	—	—	—					

SUMMARY

22,030 serums were tested by the Kahn precipitation test and by the New York City Department of Health Wassermann method.

In single bleedings from 1,621 advisory cases from the Venereal Diseases Clinics the Kahn and Wassermann tests agreed in diagnosis, as positive, doubtful, or negative, in 85% of those tests.

In repeated bleedings from 915 cases undergoing treatment, the case diagnosis agreed in only 49% of the cases. Of the 51% variations of 62% of them could be explained as being at the beginning or ending of a curve, while for 38% of the variations we could find no explanation in the absence of clinical data.

In repeated bleedings from 788 cases at Kingston Avenue Hospital (3,359 bleedings) for which we received clinical data, the Kahn and Wassermann case-diagnoses agreed in 100% of those cases clinically diagnosed as active syphilis.

In serologic Syphilis, by which is meant cases having no clinical symptoms of syphilis, but having a positive Wassermann reaction, the agreement of the Wassermann and Kahn case-diagnoses was only 70%. The syphilitic cases were all under treatment.

In syphilis and gonorrhoea, not classified as to stage of either disease, the agreement was 95.5%.

In gonorrhoea not complicated by syphilis the agreement of Kahn and Wassermann was less than in the syphilitic cases, but was greater than in the unclassified list of tests of specimens from the Venereal Disease Clinics.

A close examination of the Wassermann and Kahn curves of 788 cases, of which an excerpt is given in our Table IV, shows the variations in the Wasserman tests throughout the curves and the Kahn tests throughout the curves to be approximately the same. When the Wassermann test curve was irregular the Kahn was usually irregular. The irregularities occurred more often in the latter stages of disease.

In the attempt to evaluate these comparative tests, we must keep in mind the fact that the Kahn tests were made with an antigen very strongly reinforced with cholesterin, (0.6%), whereas, the Wassermann tests were made with a crude alcoholic extract antigen containing no cholesterin. Thus, the Kahn tests had the advantage of a much more sensitive antigen. Had the Wassermann tests been made with the Kahn antigen, the variations in the treated cases might have been less. Our percentage of agreement of diagnosis, positive, negative and doubtful, is greater than Kahn's in his latest report.[3]

CONCLUSIONS

Our study shows a strong similarity between the Kahn and Wassermann tests, regardless of the fact that the Kahn antigen was more sensitive.

Most of the variations of single tests could be explained upon the fact of the more sensitive Kahn test detecting antibody earlier in the curve and later in the curve. A small percentage of tests in which the Wassermann test was positive and the Kahn test negative was not explained by any of the tests in our study.

Our tables show the difficulties in drawing accurate conclusions in regard to the relation to stage of disease of Wassermann and Kahn tests when single bleedings from treated cases are considered. Ones should have repeated bleedings over a period.

References:

[1] Kahn, Serum Diagnosis of Syphilis, 1925 ed.

[2] Park & Williams, Pathogenic Microorganisms, 1924 ed., p. 278.

[3] Kahn, Jour. A. M. A., 1927, Vol. 89, No. 2.

THE LIMITS OF ACCURACY OF HEMOLYTIC TITRATIONS

John Koopman

The precise titration of the hemolytic reagents is a matter of fundamental importance in complement fixation work.

The fact that it is so difficult to obtain agreement among several laboratories in the results of Wassermann tests is due in a large measure to complement variations.

The unit of complement as determined by the customary titrations is a variable and indefinite quantity. It is not uniform in all laboratories and it is not uniform in any one laboratory from day to day.

As the result of an effort to increase the accuracy of complement titrations two methods of titrating complement have been devised. Although both of these methods are accurate, they may still produce different results from the same sample of complement. Because of this fact it is possible to show that titrations alone cannot be depended upon to control complement fixation tests, so that the results will be free from variations.

One of the reasons why the complement unit as at present determined is not constant is due to the fact that we cannot control the density of the cell suspension.

In order to show how variations in the density of the cell suspension produce a variable complement unit it is only necessary to prepare a simple titration such as the following:

Place a unit of complement and an excess of amboceptor, say two units, in each of seven or eight tubes. Add graded amounts of cells to these tubes so that the first will receive less than the customary amount and the last more than the customary amount with intermediate amounts in between. Balance with saline so that all tubes contain the same volume and incubate. Several tubes will probably show complete hemolysis and several incomplete hemolysis. All of these tubes contain exactly the same amount of complement and amboceptor; notwithstanding this fact it will be noted that whether a tube shows complete hemolysis depends upon the amount of cells present rather than upon the amount of complement.

This fact has been universally recognized and because the complement unit is based upon the hemolysis of a definite quantity of cells, numerous attempts have been made to standardize the cell suspension.

There is no practical method of preparing cell suspensions which can be guaranteed to produce uniform results in the hands of different workers. In fact it is very difficult for any one laboratory to control the uniformity of its own cell suspension from day to day.

The various methods which have been proposed to overcome this difficulty will not be discussed here, because the following procedure will show that the error caused by variations in the cell suspension would not be overcome even if it were possible to prepare a uniform cell suspension from day to day.

By means of a 1 c.c. pipette graduated in .1 c.c. place .1 c.c. of 5% cells in each of 10 tubes. Add an equal amount of water to each tube so as to hemolyze the cells. If the resulting solutions in the 10 tubes are compared in a colorimeter the results will show that the amount of cells delivered by each division varies. This variation is due to two factors, one is the unavoidable technical error and the other is the sendimentation of the cells in the pipette.

The last tenth c.c. contains fewer cells than any other tenth and the slower the pipetting is done the greater will be the variations between the first and the last tenth. This being so, it becomes necessary to make a change in the method of titrating complement so that the error caused by these unavoidable cells suspension variations will be automatically eliminated.

It was thought at one time that the error due to variations in the cell suspension was neutralized in complement fixation tests if the same cell suspension was used for the test as was used for the titration of the complement. This is not so because if a weak cell suspension is used in the titration it will require less complement to bring about complete hemolysis. Under these circumstances the unit will be smaller and less complement will be used in the fixation reaction itself. Therefore a weakly positive serum which fixes a small amount of complement may nevertheless fix all the complement. It would therefore be reported as giving a stronger reaction than it is entitled to. On the other hand if a denser cell suspension is used to titrate complement, more complement will be added to the fixation tests and therefore some strongly positive specimens may be reported as weakly positive.

A method of estimating complement strength which gives constant results regardless of cell suspension variations may be demonstrated as follows:

If two suspensions of sensitized cells are prepared one of which would be considered slightly less and the other slightly greater than 5% we will have two suspensions which represent the maximum variation which is likely to occur from day to day or which may occur in different laboratories.

Prepare two complement titrations in the customary manner and use the light cells in one and the heavy cells in the other. At the end of one hour's incubation it will be found that the hemolytic unit will vary in the two titrations.

The heavier cells will require slightly more complement to produce complement hemolysis.

Allow the cells in the tubes which show incomplete hemolysis to settle and compare the hemolysis in these tubes. It will be found that the corresponding tubes in each titration will show the same amount of hemolysis. The amount of cells added to one set was greater than the other and the amount of cells remaining is greater in one set than the other but the amount of hemolysis is unaffected.

With the aid of these titrations we can form an estimate as to the relative vlaue of the information furnished by an incompletely hemolyzed tube as compared with a completely hemolyzed tube.

When hemolysis proceeds until all the cells present in a tube have given up their hemoglobin it is correct to say that the reaction is complete. It is complete however only as regards the cells because there may be present in the solution some complement and amboceptor which would produce more hemolysis if more cells were present to be acted upon.

The titration above shows that complete hemolysis may be brought about in several ways. A tube containing a little more complement and more cells and another containing less complement and fewer cells will both show complete hemolysis and thus indicate a false unit. In addition to the variation of the unit caused by the cell variation another difficulty is raised when we try to determine whether hemolysis is just complete or not quite complete. All that can be said of a completely hemolyzed tube is that the reagents present are sufficient to hemolyze the cells present. The actual amount of either cannot be definitely determined.

An incompletely hemolyzed tube gives much more definite information.

A tube which contains a small amount of complement in the presence of an excess of amboceptor and cells will not hemolyze completely. Under these circumstances the reaction comes to an end only when the complement has produced all the hemolysis it is able to produce. Therefore if we stop using the point of complete hemolysis as a guide for the complement unit and use the actual amount of hemolysis produced as our index of complement strength, one of the sources of error will be eliminated.

The above method of estimating complement strength is an excellent one when it is desired to compare two complements for their relative activity. When it is used to determine the absolute strength of an unknown complement however it exhibits a serious discrepancy.

There is no universal permanent standard which can be used from day to day or place to place to show how much hemolysis a definite amount of complement should produce. Under these circumstances it would be necessary to depend upon the eye alone in judging whether the complement produced too little or too much hemolysis. This would be introducing as great an error as existed in the first place.

Therefore an addition must be made to the above method so that any degree of hemolysis may be expressed in terms having the same significance to all workers, notwithstanding the fact that no inter-laboratory comparison is possible.

The following titrations lead up to such a method of indicating complement strength.

Place .05 c.c of 10% complement in each of 6 tubes. To the first tube add .25 c.c. of salt solution, to the second .3 and so on to the last tube which receives .5 c.c. Add to each tube .1 c.c. of 5% cells and .15 c.c. of amboceptor.

We now have 6 tubes which contain the same amount of all reagents, the only difference being in the volume employed.

Incubate these tubes until the reaction comes to an end and centrifuge so that the amount of hemolysis in each may be compared.

It will be seen that the amount of hemolysis produced by .05 c.c. of complement depends upon the volume in which it acts. The amount of hemolysis in the first tube will be greater than that in the last tube, just as if different amounts of complement were present. This is partly due to the fact that the color is confined to a smaller volume in the first tube, but if the volume in all the tubes is now made uniform with salt solution it will be found that the graduations in color are still present.

Continuing with above kind of titration it will be seen that by varying the volume between still wider limits, it is possible to make .05 c.c. of complement produce any amount of hemolysis desired.

With a sufficiently large total volume, .05 c.c. of complement produces no hemolysis whatever. With a very small volume the hemolysis is so active that it will be necessary to use a heavier cell suspension if the complement is to show its full effect before all the cells are used up.

This being so it is evident that the amount of hemolysis produced by complement does not depend upon the actual amount of complement present but upon its concentration. This fact has an important bearing on the titration of complement.

Quantitative hemolysis takes place in accordance with the laws of mass action. This fact permits the activity of any complement to be accurately determined and the result stated in terms of definite significance.

As a result of a series of tests made with various concentrations of the same complement the following figures were obtained. The readings were made with a colorimeter.

Two dilutions were made from the same sample of complement, one 10% and the other 8%. A comparison was made of the amount of hemolysis produced by an equal quantity of each dilution. .2 c.c. of the 10% complement produced twice as much hemolysis as did .2 c.c. of the 8% complement, the total volume in each tube being 4 c.c.

This shows that the two complement dilutions which differ by 25% in actual concentration may differ by 100% in the amount of hemolysis they produce.

Likewise, if the concentration of any complement is doubled, the amount of hemolysis it is then able to produce is multiplied by three or four or some greater number.

There are two ways in which the concentration of complement may be varied, one is by varying the amount of complement in a given volume of solution and the other by using a given amount of complement and varying the volume of solution in which it acts.

The extent to which the amount of hemolysis is changed when the concentration of complement is raised or lowered may be used as a guide to the strength of the complement. For instance, using the customary 10% complement, .2 c.c. will probably produce 5 times as much hemolysis as .1 c.c in a total volume of 4 cc. That is, doubling the concentration increases the hemolysis 5 times.

If we prepare a weaker solution of this complement by making an 8% dilution and then repeat the above test we find that .2 c.c. of this weaker dilution may produce 6 times as much hemolysis as .1 c.c. in a volume of 4 c.c. That is, doubling the concentration of the weaker complement changes the amount of hemolysis 6 times whereas doubling the stronger complement changes the hemolysis 5 times.

If we make the complement still stronger by preparing a 12% dilution from the original stock, the hemolysis will only be increased 4 fold if we double the concentration by using a .2 c.c. and .1 c.c. in the same volume.

The amount by which the hemolysis is increased when the concentration is doubled varies very nearly inversely as the strength of the complement.

That is if we have at hand two samples of complement of unknown activity one of which produces 6 times as much hemolysis when its concentration is doubled and the other produces 3 times as much when its concentration is doubled, then the latter sample is for all practical purposes twice as strong as the former or as 3 is to 6.

In any given volume of solution and two given amounts of complement, there is but one strength of complement which will produce a given difference of hemolysis.

Therefore any complement solution which shows an agreed difference of hemolytic activity when its concentration is altered by an agreed amount has an agreed titre.

If the above statements are true, the activity of any complement may be stated in figures having the same significance to all workers and complement of any desired hemolytic activity may be prepared at will.

The relation of volume to hemolysis is worthy of further study. A reason for the fact that the amount of hemolysis varies with the concentration of the complement and not with the actual amount used, can be deduced from the following titration.

Prepare a series of 8 tubes, in the first tube put .06 c.c. of 10% complement, increase the amount by .02 c.c. until the last tube receives .20 c.c. Add sufficient salt solution to make the volume in each tube 2.5 c.c. Then add sufficient sensitized cells to make the volume 5 c.c. in each tube and incubate to completion of the reaction.

Remove the cells which remain unhemolyzed and compare the color of the solution in the several tubes by means of a colorimeter.

A titration of a certain complement made in this manner gave the following readings:

Amount of complement........	.06	.08	.10	.12	.14	.16	.18	.20 cc.
Colorimeter reading	none	none	30	15	10	7.5	6	5

Inasmuch as the colorimeter gives a smaller figure as the color increases, the figures must be reversed so that increasing hemolysis will be represented by increasing numbers. When this is done the relative amount of hemolysis in the tubes is as follows:

Complement	.06	.08	.10	.12	.14	.16	.18	.20 cc.
Hemolysis	0	0	5	10	15	20	25	30

It will be seen that .2 c.c. of complement gave a color of 30 which is 6 times as much as was produced by .1 c.c. It will also be seen that the difference in hemolysis for each .02 c.c. of complement is a constant of 5. If each reduction of .02 c.c. of complement changes the hemolysis by 5, then a reduction of .02 c.c. from the .1 c.c. tube should reduce the hemolysis from 5 to zero.

That this reasoning is correct is shown by the fact that .08 c.c. of complement did not produce any hemolysis whatever.

This means that .08 c.c. of this complement was used up in some manner without producing any hemolysis. It also means that regardless of how much of this complement was present in any tube the first .08 c.c. of it did not produce any hemolysis. Whatever hemolysis is produced in any tube is the result of the amount of complement which is in excess of .08 c.c. The tube containing .1 c.c. of this complement therefore has only .02 c.c. available for hemolysis. The same thing holds true for the other tubes as follows:

Total complement added..........	.06	.08	.10	.12	.14	.16	.18	.20
Available for hemolysis..........	0	0	.02	.04	.06	.08	.10	.12
Amount of hemolysis produced...	0	0	5	10	15	20	25	30

The amount of hemolysis produced is in direct proportion to the amount of complement available for hemolysis and not to the total amount present.

If we apply this method to any complement it will be found that by a comparison of the amount of hemolysis produced in any two tubes it is possible to calculate the activity of the complement. An example of the calculation is as follows:

In a volume of 5 c.c. .1 c.c. of complement produced a color of 5 and .16 c.c. produced a color of 15.

Let A represent the large amount of complement
Let B represent the small amount of complement
Let X represent the greater hemolysis complement
Let Y represent the smaller hemolysis complement

$$B - \frac{(A-B)\ Y}{X-Y} = \text{The concentration of the complement}$$

Substituting figures for the 2 tubes of the above titration we find

$$.1 - \frac{(.16 - .1)5}{15-5} = .07$$

The figure obtained by this calculation is a significant one for the following reason. It will be found that it always represents the largest amount of complement which can be added to sensitized cells without producing any hemolysis. This figure varies directly as the actual strength of the complement.

Two complements which give 7 and 8 as a result of such a test stand in this relation to each other in actual strength.

Sensitized cells seem to require a definite concentration of complement before hemolysis begins.

If the concentration of complement in a tube is less than the required minimum no hemolysis takes place although the actual amount of complement present may be a considerable one.

If the concentration is increased it will finally reach a point where the cell resistance will be overcome and hemolysis will begin.

This required concentration of complement being a constant, the activity of any complement may be determined by finding how much or how little of it can be added to a definite volume of solution before hemolysis begin.

Any complement can be brought to an agreed standard strength by changing its dilution until a definite amount in a definite volume will just do this.

In order to titrate complement by this method it is only necessary to prepare a 10% dilution of the unknown and set up six tubes as follows:

Complement	.01	0.15	.02	.025	.03	.035 cc.
Salt solution to make the volume in all tubes...	.3 cc.					
Sensitized cells	.2	.2	.2	.2	.2	.2 cc.

The largest amount of complement which produces no hemolysis is a direct indication as to the activity of the complement.

Experience has shown that six times this amount corresponds to the amount of complement we aim to use when we use 2 units as determined by the classical method of titrating.

This method far surpasses the classical titration method in accuracy for several reasons.

1. The only tube in a titration which varies exactly as the strength of the complement is the tube which shows no hemolysis.

2. It is much easier to determine a point of no hemolysis than it is to determine the point of complete hemolysis.

3. When complete hemolysis is read the number of cells present makes a difference in the result. This is not the case when the point of no hemolysis is read.

Additional reasons in favor of using the point of no hemolysis as a guide are the following:

Colorimeter readings will show that when any tube shows complete or almost complete hemolysis of a fairly dense cell suspension such as is used in ordinary titrations, relatively large increases or decreases in the amount of complement will produce relatively small changes in the amount of hemolysis. This is if we use a strongly hemolyzed tube as a standard by which to judge complement strength, large variations in the amount of complement may pass unnoticed because they produce such small changes in the amount of hemolysis. This is a very important cause of error in judging the ordinary complement unit.

If we judge the amount of complement by the amount of hemolysis in a tube which is only partly hemolyzed or not at all hemolyzed the conditions are entirely different, in this case a very slight increase or decrease in the amount of complement will produce a magnified difference in the amount of hemolysis. The chances for accuracy are therefore all on the side of the method which uses slightly hemolyzed or unhemolyzed tubes as guides.

A simple method of showing this to be so is to add .1 c.c. and .15 c.c. of complement to two tubes. Add .75 c.c. of sensitized cells and .65 c.c. of salt to each.

The hemolysis will be almost complete and both tubes will be very nearly alike, as to color. Using the same amount of complement and sensitized cells in two duplicate tubes add 3.5 c.c. of salt to each incubate and compare the color. Because of the larger volume the second pair of tubes will show little hemolysis but the difference between them will be great. When the hemolysis is great, 50% difference in the amount of complement produces about 20% difference in the amount of hemolysis. When the hemolysis is small 50% difference in the amount of complement makes more than 100% difference in the amount of hemolysis.

It was stated in the beginning of this article that several methods for accurately titrating complement would be given and that their very accuracy could be used to show that titrations alone cannot be depended upon to produce accurate fixation results.

The above method is one of these and the reason it does not guarantee accurate fixation results is as follows:

If a sample of complement is titrated with the cells of several sheep it will be noticed that occasionally the titre will show a marked variation with the cells of some one sheep. This variation will also occur when fresh cells are compared with cells which are several days old even though they are from the same sheep.

The cells of different sheep vary markedly in their resistance to the action of the hemolytic reagents. If we adjust the complement in accordance with the cells of each sheep a different amount of complement might be used in each instance.

Under these circumstances although the above method corrects one error peculiar to the customary titration and that is the error due to differences in cell suspension density, it still retains the error due to variations in cell resistance.

If the cells are less resistant than normal, the complement will appear stronger than it really is because of the increased hemolysis. If the strength of the complement is reduced and used in a fixation reaction, each specimen will receive less complement than usual and less complement need be fixed in order for the test to show complete fixation.

Any method of estimating complement strength which depends upon the amount of hemolysis produced will always be subject to this type of error. Increasing the accuracy of such titrations does not help us to determine the actual amount of fixable complement.

A Titration of Actual Complement Strength

This method provides for the estimation of complement strength in terms of fixable units regardless of the fact that such a complement may produce more or less hemolysis with cells of different sheep or cells of varying resistance.

Of all the reagents used in hemolytic work, amboceptor is the most stable. It retains its titre over relatively long periods of time. For this reason if a definite dilution of a particular amboceptor is made daily it furnishes a standard which can be used to determine how much the other reagents vary. If, for example, .1 c.c. of a 1 to 1,000 dilution of a certain amboceptor produces more hemolysis one day than another we know that the difference is due to the other reagents and not to the amboceptor. The difference may be due to the complement used or to the cells or to a combination of both.

If that part of the difference which is due to the variation in complement strength can be subtracted from the total the remaining difference will be due to the cells alone.

The variation in hemolysis which is due to variation in complement strength can be neutralized or allowed for.

The following titrations will show this to be so.

THE QUANTITATIVE RELATIONSHIP OF COMPLEMENT TO AMBOCEPTER

Variations in the strength of the ambocepter used in complement titrations and variation in the strength of the complement used in amboceptor titrations can be prevented from affecting the results of such titrations if the time factor in reading is removed.

That is, it makes no difference whether the amboceptor used to titrate complement varies considerably if the titration is read at the end of its reaction time instead of after a definite number of minutes.

To illustrate this point prepare two complement titrations which are identical except that one is completed with a dilution of amboceptor which is considered fast and one with a dilution which is considered slow. If these titrations are read after ten minutes incubation they will read differently because of the difference in amboceptor. After 20 minutes the difference will be less but still considerable. If they are read after both have reached the point where hemolysis ceases, the results will be absolutely alike. The end point for one may be 45 minutes and for the other 75 minutes or more.

If the conditions are reversed and amboceptor is used in small amounts in the presence of an excess of complement the same thing may be said of complement as was said of amboceptor in the above titration.

As a result of the information furnished by these titrations the following statement is justified.

Variation in the excess of amboceptor in a complement titration or variation in the excess of complement in an amboceptor titration changes the time required for the reaction to reach completion. It does not change the end point. This means that in titrating an unknown complement, any variation in the strength of the amboceptor such as was used above or such as may occur in different laboratories will have no affect on the result provided that a reading is taken when the action has ceased and not at the end of any definite number of minutes. Furthermore the result can be accepted with greater certainty if the excess of amboceptor is reasonably large and the time correspondingly short, making the end point more definite.

With any combination of complement and amboceptor, the amount of possible hemolysis is limited by the reagent which is present in the lesser amount while the time is controlled by the one present in the greater amount.

THE COMPENSATION OF AMBOCEPTOR FOR COMPLEMENT

If we prepare two tubes, one containing a small amount of complement, say .02 c.c. with an excess of amboceptor say .07 c.c. and the other containing amboceptor say .02 c.c. and an excess of complement say .07 c.c., they may both show the same amount of hemolysis.

This fact has led to the erroneous impression that a lack of one reagent is compensated for by an excess of the other.

The reason these two tubes show an equal amount of hemolysis is that .02 c.c. of complement can only do a certain amount of work regardless of the amount of amboceptor present and when its work is done any amboceptor in excess of the amount required by the complement remains in the tube. The same is true of .02 c.c. of amboceptor which is able to do a definite amount of work and when this is done any excess of free complement is left in solution. The equal amount of hemolysis produced is not an evidence of compensation.

If a lack of complement was compensated for by an excess of amboceptor we would be able to make .05 cc. of complement produce as much hemolysis as .07 c.c. of complement by adding more amboceptor to .05 c.c. of complement.

The amount of hemolysis which a definite amount of complement is able to produce cannot be increased by adding more amboceptor. It can be decreased by adding an insufficient amount of amboceptor, but in this case the hemolysis is limited to the amount which the amboceptor can produce and the complement is then in excess.

Another fact concerning the relationship of complement and amboceptor is that neither reagent is able to produce its maximum hemolysis unless there is present an excess of the other. One reason for this is the change in the reaction time caused by an excess of either reagent. The smaller the excess the greater the time necessary for the reaction to reach completion and when there is no excess of either the time is infinite and a small amount of each reagent remains unused. This explains why a unit of a complement plus a unit of amboceptor does not produce complete hemolysis although either one can do so if an excess of the other is present.

There is no danger of a complement titration hemolyzing too fast or too far as the result of an excess of amboceptor, in fact the end point is most easily determined when the excess is reasonably large and the time short.

We know that if an excess of complement is used in the presence of a small amount of amboceptor, the actual strength of the complement does not affect the amount of hemolysis produced by the amboceptor, but alters the reaction time only.

It must follow therefore that if the amboceptor is kept at a constant dilution and the complement, cells and time factor are in excess, any variation in the amount of hemolysis from day to day is due only to the resistance of the cells.

Advantage may be taken of these facts to titrate complement to an exact fixable standard. We can adjust the strength of the complement by means of the constant amboceptor and thus discount the variation in the resistance of the cells.

An example of such titration is the following:

Choose a dilution of amboceptor which will contain 2 units in each .1 c.c. and consider this dilution arbitrarily as a standard.

.05 c.c. of such a dilution is allowed to hemolyze with .15 c.c. of 10% complement, and .2 c.c. of cells. The final volume in the tube should be made up to 1 c.c. with saline. This tube will vary in hemolysis from day to day only if the cells vary in resistance and for no other reason. Variations in complement strength do not affect it.

Prepare a 10% dilution of the complement to be titrated and place various amounts in a series of tubes as follows:

.035 .040 .045 .050 .060 cc.

Add an excess of amboceptor and cells and equalize with salt to a volume of 1 c.c. Incubate these tubes and the amboceptor tube mentioned above, to the completion of the reaction. Centrifuge all of the tubes. The color of the solution in the amboceptor tube is compared with the color in the complement tubes.

The tubes containing various amounts of complement will produce an amount of hemolysis which, as has been explained above, will depend upon the activity of the complement modified by the resistance of the cells.

Some one of these tubes will produce an amount of hemolysis which will be the same as the amount of hemolysis produced in the amboceptor tube.

The amount of hemolysis in the amboceptor tube may vary with the resistance of the cells, but whatever hemolysis does take place is always produced by the constant amount of amboceptor. The complement tube which produces the same amount of hemolysis as the amboceptor tube is also affected by the cell resistance to the same degree. This tube however contains a standard amount of complement because the same cells resistance is common to all the tubes and we have compared the complement directly with the stable amboceptor.

Under this plan the fixable amount of complement can be accurately determined regardless of the fact that it may produce more or less hemolysis, with different cell suspensions.

Either of the two methods given above for the titration of complement will produce accurate results from its own point of view but each method may produce a different result from the same sample of complement.

From a consideration of these titrations it is evident that complement may be titrated for its hemolytic value or for its actual fixable quantity, but neither method will guarantee accurate fixation results.

If we add to our complement fixation tests that amount of complement which produces a definite amount of hemolysis we may be adding different fixable units.

If we add a standard fixable amount of complement to the tests regardless of the amount of hemolysis it is able to produce, the cells of some particular sheep may cause the amount left for hemolysis to appear greater or less than it really is and thus lead to false conclusions as to the strength of the reaction.

OSMOSTIC PRESSURE AND ITS RELATION TO SPECIFIC AND NON-SPECIFIC HEMOLYSIS

John Koopman

The fact that small changes in the tonicity of the hemolytic reagents cause a considerable change in the amount of hemolysis they produce, led us to investigate the role of osmosis in specific and nonspecific hemolysis.

Water added to a asuspension of erythrocytes causes them to lose their hemoglobin. Exactly what happens to the cell itself is not clearly understood.

Recent text books on physiology state that the cell takes up water and increases in volume until it finally bursts from the effect of the increased internal pressure.

The results of a number of simple experiments with cell suspensions and water seem to indicate that the cells do not swell up and do not burst but remain intact during and after hemolysis.

If we prepare a number of salt solutions, starting with .8% and decrease the percentage by .025% until we reach .525%, their effect on sheep cells will provide data of some interest.

Place 1 c.c. of each solution in a series of twelve tubes. To each tube add a small amount of a rather heavy suspension of cells in serum or in isotonic salt solution. .05 c.c. or 1 drop of the cell suspension will be sufficient to produce the effect desired. Hemolysis will probably take place to a very slight extent in the .75% or the .7% tube. Each succeeding tube will show a larger amount and the .525% tube may show complete hemolysis.

After standing about one-half hour the cells and salt solution will have reached their equilibrium and no further hemolysis will take place. The remaining cells will settle.

If we hold to that explanation of hemolysis which supposes the cells to burst when they give up their hemoglobin under reduced pressure, we must say that in the first tube some of the cells have burst and some have remained intact. In each succeeding tube a still larger number of cells must have burst until all have been destroyed.

If some cells are presumed to be more fragile than others we must believe that there is a gradation in fragility among cells which causes them to break up in regular order under uniformly reduced pressure. This of course is not possible.

Proceeding with the above experiment, the supernatant fluid in each of the tubes is poured off after the cells have settled. Divide the fluid from each tube into two parts and keep one set of tubes for control. To each tube of one set add another drop of the original suspension of cells. These new cells will again partly hemolyze to almost the same extent as the first cells did and will almost double the color of the fluid. That is, partial hemolysis again takes place in the tubes which could not hemolyze any more of the first cells added.

A reasonable explanation of what has taken place is this: When cells are added to a hypotonic salt solution each cell promptly adjusts itself to the new condition by dialyzing hemoglobin until the pressure within the cell has fallen to that of the fluid in which it is suspended. This means that if a small amount of cells is added to a hypotonic salt solution, the solution can not take from these cells enough hemoglobin to increase its tonicity to normal but only enough to reduce the cells to the tonicity of the solution. When the cells have lost enough hemoglobin to reduce their internal pressure to that of the surrounding medium, no further hemolysis can take place although the fluid surrounding them is still hypotonic. In order for the fluid to withdraw more hemoglobin more normal cells must be added. These in turn will only give up enough hemoglobin to adjust themselves to the new tonicity of the salt solution.

Within certain limits, the greater the number of cells added the deeper will be the color of the solution because a greater number of cells must lose some of their hemoglobin in adjusting themselves to the new condition. This loss of hemoglobin will take place upon each addition of normal cells until the hemoglobin released has raised the tonicity of the fluid to that of a normal cell.

Although the cells may have lost part of their hemoglobin there has been no rupture or other injury to them. This will be found to be so if the cells remaining in any tube are resuspended in salt solution of normal tonicity; they will then act exactly like normal cells. In the case of complete hemolysis in water each cell loses all its hemoglobin before reaching equilibrium with the external fluid.

The adjustment of a cell to its surroundings takes place very quickly. It takes place in a very short time when the pressure of the fluid outside is greater than that of the hemoglobin inside, but when the pressure outside is smaller the adjustment is exceedingly rapid. A simple experiment will illustrate this.

If cells suspended in normal salt solution are added to a salt solution two or three times normal they will float because they are so much lighter, but in a very few minutes they will have made the necessary exchange and begin to settle just as they do in normal saline and at about the same rate.

If on the other hand cells are suspended in 3% salt solution and an amount of water is added which will just dilute that solution to normal, it is almost impossible to mix the two without some of the cells hemolyzing.

When the fact is taken into consideration that dilute salt solutions such as these are known to reach equilibrium with almost incredable rapidity when mixed, it will be realized how quickly the cell must react in order to hemolyze before the stronger salt solution mixes with the water.

If cells suspended in strong salt solution are poured into another solution of normal tonicity which has been made by mixing the 3% salt solution with enough water to bring it to normal, no hemolysis takes place. This shows that it is not the shock of being poured into a solution of lower tonicity which causes the hemolysis, but the fact that the cells are affected by the water before the stronger salt can diffuse into it and raise it to normal.

Osmotic Pressure and Specific Hemolysis

The sheep which we keep on hand to supply us with blood for our Wassermann work, sometimes develop a varying resistance to our specific reagents. This variation among different sheep formerly caused considerable trouble in our complement titrations and fixation work. Complement kept frozen from day to day would sometimes appear to have become much stronger or much weaker because of the varying resistance of the cells of the several sheep. Upon numbering the sheep and keeping records we were finally able to predict just what effect each sheep would have upon the titre of complement and thus avoid surprises due to the apparent change of strength of our reagents.

On making tests of each sheep's blood with salt solutions of varying tonicity as outlined above, we found that the cells which showed some hemolysis with .775% salt solution also hemolyzed rapidly with our hemolytic reagents and made the complement appear to be very active. Cells which showed no hemolysis until the salt solution was reduced to .7 or less also resisted hemolysis with the hemolytic reagents and caused the complement to appear weaker.

Because of this apparent relation between osmotic pressure and specific hemolysis, the effect of salt solutions of various percentages upon specific hemolysis was investigated. It was found that the difference between the amount of hemolysis obtained by using all reagents made up with .9% salt as compared with .8% salt is considerable, and that the amount of hemolysis can be controlled by altering the tonicity of the salt solution.

If it is possible to change the amount of hemolysis produced by the hemolytic reagents by altering the tonicity of the salt solution osmosis must play a large part in specific hemolysis as well as in water hemolysis.

When partial hemolysis is produced by the hemolytic reagents we have no more reason for saying that some of the cells have broken up and some have not than we have for saying this when hypotonic salt solution produces the same effect. Dialysis of hemoglobin seems to be the controlling factor in both. Cells which are partly hemolyzed specifically may be resuspended in stronger salt solution and they will show no visible injury.

If the hemolysis produced by the hemolytic reagents is due to osmosis where has the change in pressure taken place? The presence of complement and amboceptor does not reduce the tonicity of the salt solution because if it did, the erythrocytes of any animal would hemolyze with any amboceptor. The presence of complement and a specific amboceptor must therefore raise the internal pressure of the erythrocytes. The cell then dialyzes hemoglobin until the internal pressure falls to normal again.

If we add .05 c.c. of 10% complement to sensitized cells in the presence of salt solutions of increasing tonicity from .9% up, the first tube will probably show complete hemolysis. Each succeeding tube will show a smaller amount of hemolysis until a point is reached where no hemolysis takes place. The series will look exactly like a titration when varying amounts of complement are used. In fact complement may be titrated with perfectly satisfactory results by altering the salt solution instead of complement.

The condition may also be reversed and varying amounts of complement added to tubes containing salt solution of double physilogical strength. The largest amount of complement which can be added without producing any hemolysis will be an index of its potency.

We have found that it requires a salt solution of somewhere near 1.7% to keep .05 c.c. of 10% complement from showing any hemolysis of sensitized cells in a total volume of .5 c.c. This means that this amount of the hemolytic reagents has caused an increase of 100% in the interial pressure of the cell, because any outside pressure less than that of double strength salt will allow the hemoglobin to diffuse.

In the experiments outlined above where partial hemolysis was produced by hypotonic salt solutions, it was shown that hemolysis ceases when the tonicity of the cell has fallen to that of the surrounding fluid. The fluid is still hypotonic and is able to withdraw more hemoglobin if more cells of normal tonicity are added.

The same conditions seem to govern the amount of hemolysis produced by the specific hemolytic reagents. Assume that we have a tube which contains sufficient complement and sensitized cells to produce only partial hemolysis after prolonged incubation. It may appear that the remaining cells would hemolyze if any complement remained and that the only reason the hemolysis ceases is because the complement is exhausted.

It can be shown that the complement is not exhausted and that more hemolysis will occur if more sensitized cells are added. The additional cells cannot of course be added to the same tube. Two tubes must be prepared to show this effect, each one receives the same amount of complement. One tube then receives a certain amount of salt solution and sensitized cells, the other receives a double dose of sensitized cells and a correspondingly smaller amount of salt solution in order to keep the volume in each tube equal.

The tube containing the larger amount of cells will show the larger amount of hemolysis. An explanation of the cause of this increase is as follows.

The complement present is able to raise the internal pressure of a cell a certain amount.

The amount by which it is raised depends upon the concentration of the complement.

Although the complement present cannot raise the pressure of this cell high enough to produce complete hemolysis it still can raise the internal pressure of more normal cells to the same extent. When this is done more hemolysis is produced because more cells lose an equivalent amount of hemoglobin in returning to normal pressure. Just how far this increase of hemolysis with increase of cells will go was not determined.

The information to be gained from a comparison of the physics of hemolysis by hypotonic salt solutions and by specific reagents is this: Regardless of the nature of the changes which occur within the cell when it is placed in contact with amboceptor and complement, the hemolysis which takes place after that is due to an increase of the osmotic pressure within the cell. The amount of hemolysis which occurs is in direct proportion to the increase in the internal pressure. When we speak of partial hemolysis we do not mean that a few of the cells have hemolyzed completely and others not at all but that all of the cells have given up a portion of their hemoglobin to the same degree.

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